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Request for grant of a patent

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2.	Patent application number *(The Patent Office will fill in this part)*	0314262.7
		19 JUN 2003
		19JUN03 E816309-3 D02866 P01/7700 0.00-0314262.7
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	**The Nottingham Trent University** Faculty of Science and Mathematics Clifton Lane Nottingham NG11 8NS United Kingdom
	Patents ADP number *(if you know it)*	
	If the applicant is a corporate body, give the country/state of its incorporation	United Kingdom
		79 370 30001
4.	Title of the invention	NOVEL COMPOUNDS AND METHODS OF USING THE SAME
5.	Name of your agent *(if you have one)*	**ERIC POTTER CLARKSON** **PARK VIEW HOUSE** **58 THE ROPEWALK** **NOTTINGHAM** **NG1 5DD**
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	
	Patents ADP number *(if you know it)*	**1305010**

6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number	Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application		Date of filing *(day / month / year)*
8.	Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:* a) *any applicant named in part 3 is not an inventor; or* b) *there is an inventor who is not named as an applicant, or* c) *any named applicant is a corporate body. See note (d))*	YES		

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Continuation sheets of this form	
Description	55
Claims*(s)*	8
Abstract	1
Drawing*(s)*	19 + 19

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Priority Documents	0
Translations of priority documents	0
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	NO
Request for preliminary examination and search *(Patents Form 9/77)*	NO
Request for substantive examination *(Patents Form 10/77)*	NO
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature Eric Potter Clarkson
ERIC POTTER CLARKSON

Date
18 June 2003

12. Name and daytime telephone number of person to contact in the United Kingdom

0115 9552211

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NOVEL COMPOUNDS AND METHODS OF USING THE SAME

The present invention provides compounds and methods for using the same in medicine. In particular, the present invention provides compounds and methods for inhibiting human diseases and/or processes wherein transglutaminase-mediated protein modification occurs, such as fibrosis, scarring and cancer.

Transglutaminases (TGases) are an important class of protein crosslinking enzymes that catalyse protein aggregation reactions in blood coagulation (Greenberg, C.S., *et al.*, 1991, *FASEB J.* **5**, 3071-3077), skin maturation (Thacher, S. M. & Rice, R. H., 1985, *Cell* **40**, 685-695) and the clotting of seminal secretions (Dubbink, H.J., *et al.*, 1999, *Lab. Invest.* **79**, 141-150). The most widespread member of the family is the cellular form of the enzyme, tissue transglutaminase (tTGase), which is expressed in varying amounts in many cell types. Like the well-characterised plasma TGase (blood coagulation factor XIIIa) (Greenberg, C.S., *et al.*, 1991, *FASEB J.* **5**, 3071-3077) and keratinocyte TGase (Thacher, S. M. & Rice, R. H., 1985, *Cell* **40**, 685-695), tTGases are calcium-dependent enzymes that catalyse the formation of crosslinks proteins via ε(γ-glutamyl) isopeptide bonds and the incorporation of polyamines at certain glutamine residues (Greenberg, C.S., *et al.*, 1991, *FASEB J.* **5**, 3071-3077). However, tTGase is unique in the transglutaminase family of enzymes in that is able to bind and hydrolyze GTP and ATP (Achyuthan, K. E. & Greenberg, C. S., 1987, *J. Biol. Chem.* **262**, 1901-1906), and to

bind to fibronectin (Achyuthan, K. E., *et al.*, 1995, *J. Immunol. Methods* **180**, 67-79).

Tissue TGase is predominantly located in the cytosol, although tTGase has also been reported to exist in the nucleus (Lesort, M., *et al.*, 1998, *J. Biol. Chem.* **273**, 11991-11994), at the cell surface and in the extracellular matrix (Martinez, J., *et al.*, 1994, *Biochemistry* **33**, 2538-2545). The enzyme is highly expressed in endothelial cells (Greenberg, C. S., *et al.*, 1987, *Blood* **20**, 702-709) and its activity at the surface of such cells is thought to enhance basement membrane stabilisation, cell spreading and cell adhesion (Martinez, J., *et al.*, 1994, *Biochemistry* **33**, 2538-2545; Greenberg, C. S., *et al.*, 1987, *Blood* **20**, 702-709; Kinsella, M. G. & Wight, T. N., 1990, *J. Biol. Chem.* **265**, 17891-17896; Jones, R.A., *et al.*, 1997, *J. Cell Sci.* **110**, 2461-2472; Gaudry C. A., *et al.*, 1999, *Exp. Cell Res.* **252**, 104-113). However, the overall significance of the high amount of enzyme in this cell type and its biological function is poorly understood.

Protein modification mediated by tissue transglutaminases has been implicated in the pathology and aetiology of numerous diseases and processes (see review by Aeschlimann & Thomazy, 2000, *Connective Tissue Research* **41(1)**:1-27). For example, tTGase-mediated protein modification has been shown in occur in fibrosis and scarring (Johnson *et al.*, 1999, *J. Am. Soc. Neph.* **10**:2146-2157), neurodegenerative diseases including Huntingdon's disease and Alzheimer's disease (Citron *et al.*, 1999, *J. Biol. Chem.* **276**:3295-3301), coeliac disease (Marzari *et al.*, 2001, *J. Immunol.* **166**:4170-4176), thrombosis (Ariens *et al.* 2002, *Blood* **100**, 743-754), cancer (Van Groningen *et al.*, 1995, *Int. J. Cancer* **60**:383-387; Mehta, 1994, *J. Cancer* **58**:400-406; Mehta *et al.*, 2002, *J. Natl.*

Cancer Inst. **94**:1652-1654), AIDS (Amendola *et al.*, 2002, *J. Immunol. Methods* **265**:149-159), psoriasis and inflammatory diseases of the joints (Johnson *et al.*, 2001, *Am. J. Pathol.* **159**:149-163). Tissue TGase has also been implicated in a number of diseases involving angiogenesis, such as the development of solid tumours and rheumatoid arthritis (Folkman, J., 1995, *Nat. Med.* **1**, 27-31).

Hence, tTGase represents a potential target in the development of new treatments of such diseases and disorders.

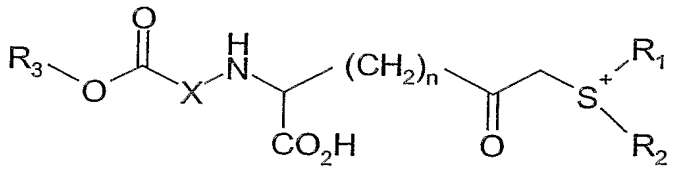
Several classes of transglutaminase inhibitor compounds are known in the art, including competitive amine inhibitors, competitive glutamine inhibitors and irreversible inhibitors. Competitive amine inhibitors include dansylcadaverines (Lorand *et al.*, 1966, *Biochem. Biophys. Res. Commun.* **25**, 629; Lorand *et al.*, 1968, *Biochemistry* **7**, 1214) and *N*-phenyl-*N'*-(ω-aminoalkyl)thioureas (Lee *et al.*, 1985, *J. Biol. Chem.* **260**, 14689). Competitive glutamine inhibitors include aliphatic amides (Gross & Folk, 1973, *J. Biol. Chem.* **248**, 1301), dipeptides (Gross & Folk, 1973, *J. Biol. Chem.* **248**, 6534) and polypeptides (Gorman & Folk, 1984, *J. Biol. Chem.* **259**, 9007). Irreversible inhibitors include iodoacetamide (Gross & Folk, 1973, *J. Biol. Chem.* **248**, 6534; Folk & Cole, 1966, *J. Biol. Chem.* **241**, 5518), phenol-containing halomethyl ketones (Folk & Gross, 1971, *J. Biol. Chem.* **246**, 6683), alkyl isocyanates (Gross *et al.*, 1975, *J. Biol. Chem.* **250**, 7693), α-halomethylcarbonyl inhibitors (Reinhardt, 1980, *Appl. Biochem.* **2**, 495), dihydroisoazoles (US 4,912,120), azoles, azolium salts (US 4,968,713, thiadiazoles (Keillor, 2001, *Biorg. Med. Chem.* **9**, 3231), and epoxides (Keillor, 2002, *Biorg. Med. Chem.* **10**, 355).

More recently, Pluira *et al.* (1992) *J. Enzyme Inhibition* **6**, 181-94 reported irreversible inhibition of transglutaminases by sulfonium methylketones (see also US 4,912,120).

The present invention seeks to provide novel transglutaminase inhibitor compounds suitable for use as therapeutic agents.

Summary of the Invention

According to a first aspect of the invention, there is provided a compound of Formula I:



I

wherein:

'X' represents an amino acid group;

'n' is an integer between 1 and 4;

'R_1' and 'R_2' each independently represent lower alkyl; and

'R_3' represents benzyl, t-butyl or 9-fluorenylmethyl.

Preferably, X is selected from the group consisting of phenylalanine, glutamine, isoleucine, alanine, glycine, tyrosine, proline, serine and glutamic acid. Thus, preferred compounds of the invention include *N*-benzyloxycarbonyl-*L*-glutamyl-γ-isopropylamide-6-dimethylsulfonium-5-oxo-*L*-norleucine bromide salt and *N*-benzyloxycarbonyl-*L*-glutamyl-γ-piperidinamide-6-dimethylsulfonium-5-oxo-*L*-norleucine bromide salt.

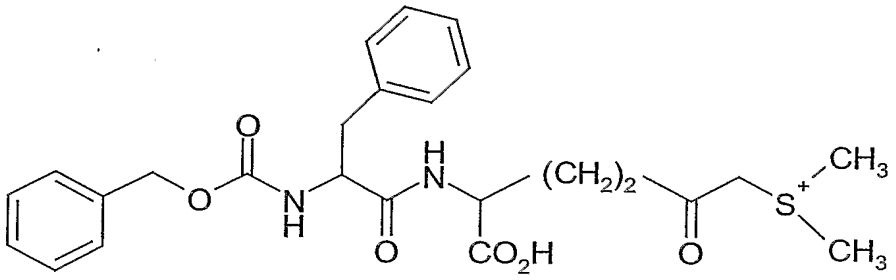
In a preferred embodiment of the first aspect of the invention, 'n' is 2.

The term "lower alkyl" is intended to include linear or branched, cyclic or acyclic, C_1-C_5 alkyl, which may be saturated or unsaturated. Lower alkyl groups which R_1 and R_2 may represent include C_1-C_4 alkyl, C_1-C_3 alkyl, C_1-C_2 alkyl, C_2-C_5 alkyl, C_3-C_5 alkyl, C_4-C_5 alkyl, C_2-C_4 alkyl, C_2-C_3 alkyl and C_3-C_4 alkyl. Preferred lower alkyl groups which R_1 and R_2 may represent include C_1, C_2, C_3, C_4 and C_5 alkyl.

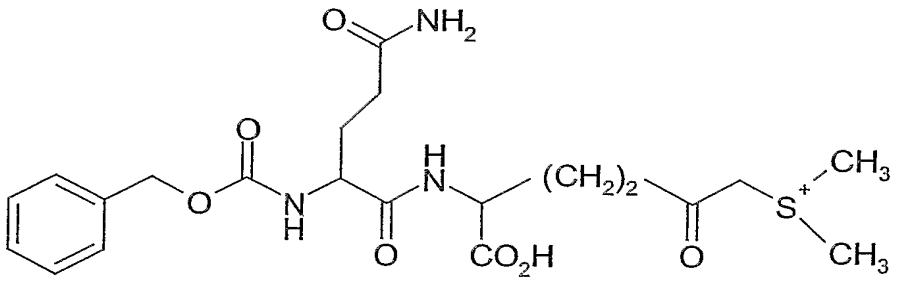
Preferably, 'R_1' and 'R_2' are $-CH_3$ or $-CHCH_2$. More preferably, 'R_1' and 'R_2' are both $-CH_3$.

Preferably, 'R_3' is benzyl.

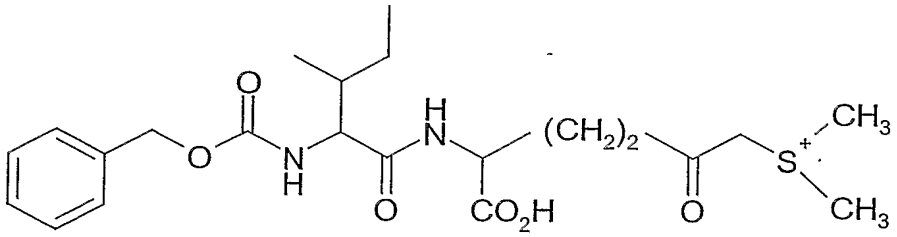
In a preferred embodiment of the first aspect of the invention, the compound is selected from the group consisting of:



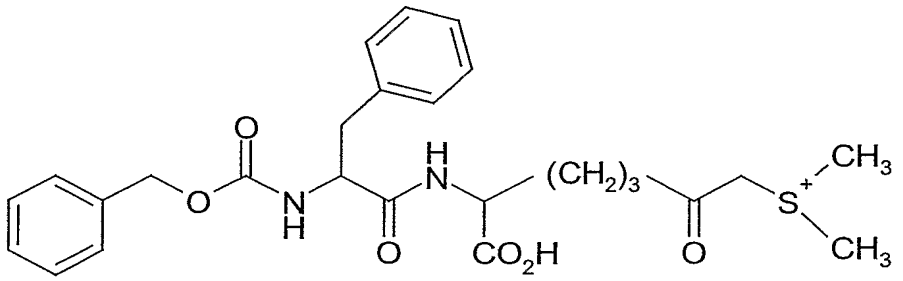
(a) *N*-Benzyloxycarbonyl-*L*-phenylalanyl-6-dimethylsulfonium-5-oxo-*L*-norleucine ("*Compound 281*")



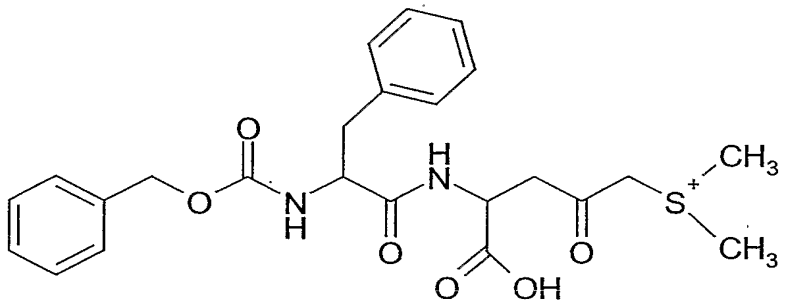
(b) *N*-Benzyloxycarbonyl-*L*-glutaminyl-6-dimethylsulfonium-5-oxo-*L*-norleucine ("*Compound 285*")



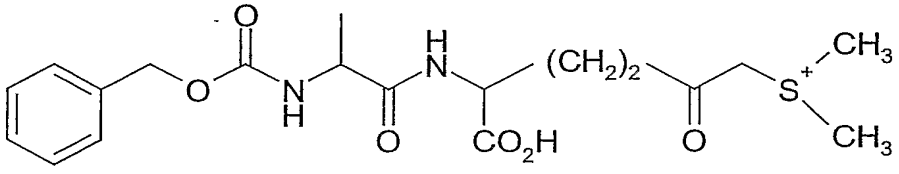
(c) *N*-Benzyloxycarbonyl-*L*-isoleucinal-6-dimethylsulfonium-5-oxo-*L*-norleucine ("*Compound 286*")



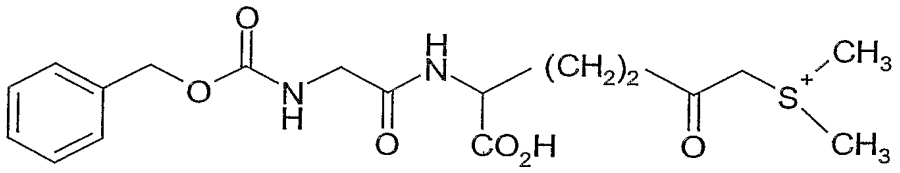
(d) *N*-Benzyoxycarbonyl-*L*-phenylalanyl-7-dimethyl-sulfonium-6-oxo-heptanoic acid ("*Compound 288*")



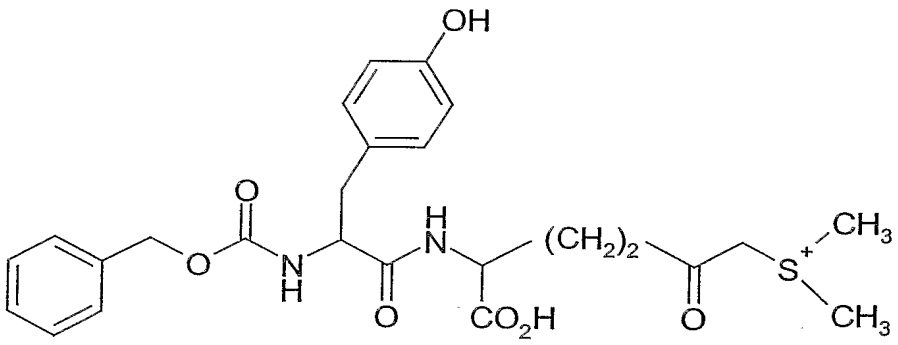
(e) *N*-Benzyloxycarbonyl-L-phenylalanyl-L-5-dimethylsulfonium-4-oxo-norvaline ("*Compound 289*")



(f) *N*-Benzyloxycarbonyl-*L*-alaninal-6-dimethylsulfonium-5-oxo-*L*-norleucine ("*Compound 291*")



(g) *N*-Benzyloxycarbonyl-*L*-glycinal-6-dimethylsulfonium-5-oxo-*L*-norleucine ("*Compound 292*")



(h) *N*-Benzyloxycarbonyl-*L*-tyrosinal-6-dimethylsulfonium-5-oxo-*L*-norleucine ("*Compound 293*")

It will be appreciated by persons skilled in the art that pharmaceutically, and/or veterinarily, acceptable derivatives of the compounds of formula I, such as salts and solvates, are also included within the scope of the invention. Salts which may be mentioned include: acid addition salts, for example, salts formed with inorganic acids such as hydrochloric, hydrobromic, sulfuric and phosphoric acid, with carboxylic acids or with organo-sulfonic acids; base addition salts; metal salts formed with bases, for example, the sodium and potassium salts.

Thus, the compounds of formula I may be counterbalanced by counter-anions. Exemplary counter-anions include, but are not limited to, halides (*e.g.* fluoride, chloride and bromide), sulfates (*e.g.* decylsulfate), nitrates, perchlorates, sulfonates (*e.g.* methane-sulfonate) and trifluoroacetate. Other suitable counter-anions will be well-known to persons skilled in the art.

Preferably, the compound is a bromide salt.

It will be further appreciated by skilled persons that the compounds of formula I may exhibit tautomerism. All tautomeric forms and mixtures thereof are included within the scope of the invention.

Compounds of formula I may also contain one or more asymmetric carbon atoms and may therefore exhibit optical and/or diastereoisomerism. Diastereoisomers may be separated using conventional techniques, *e.g.* chromatography or fractional crystallisation. The various stereoisomers may be isolated by separation of a racemic or other mixture of the compounds using conventional, *e.g.* fractional crystallisation or HPLC, techniques. Alternatively the desired optical isomers may be made by reaction of the appropriate optically active starting materials under conditions which will not cause racemisation or epimerisation, or by derivatisation, for example with a homochiral acid followed by separation of the diastereomeric esters by conventional means (*e.g.* HPLC, chromatography over silica). All stereoisomers are included within the scope of the invention.

Preferably, the compounds of the first aspect of the invention comprise L-amino acid groups.

A second aspect of the invention provides a pharmaceutical formulation comprising a compound according to the first aspect of the invention and a pharmaceutically acceptable carrier.

By 'pharmaceutically acceptable carrier' we include a substantially non-toxic, pyrogen-free excipient or adjuvant.

The formulation according to the second aspect of the invention may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient (*i.e.* a compound according to the first aspect of the invention) with the carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations in accordance with the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste. It will be appreciated by those skilled in the art that the compounds for oral administration should preferably be formulated so as to be protected in the gut and to permit bioadsorption.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose or an appropriate fraction thereof, of an active ingredient.

A third aspect of the invention provides a method of making a compound according to the first aspect of the invention comprising the following steps:

(a) reacting an *N*-α-CBZ-protected amino acid *N*-hydroxy-succinimide or *para*-nitrophenyl ester with 6-diazo-5-oxo-L-norleucine; and

(b) reacting the bromomethyl ketone produced in step (a) with dimethyl sulphide

N-α-CBZ-protected amino acid *N*-hydroxy-succinimide esters are commercially available, for example from Novabiochem (Calbiochem), Laufelfingen, Switzerland.

6-Diazo-5-oxo-L-norleucine (DON) is also commercially available (Sigma-Aldrich, Cat. No. D2141). Alternatively, DON may be synthesised, for example as described in Coutts & Saint (1998) *Tetrahedron Lett.* **39**:3243.

Preferably, step (a) comprises reacting an *N*-α-CBZ-protected amino acid *N*-hydroxy-succinimide or alternatively a *para*-nitrophenyl ester with 6-diazo-5-oxo-L-norleucine in the presence of tetrahydrofuran (THF), water and triethylamine followed by reacting the products thereof with hydrogen bromide in the presence of ethyl acetate.

Advantageously, the *N*-α-CBZ-protected amino acid *N*-hydroxy-succinimide or *para*-nitrophenyl ester is selected from the group consisting of *N*-α-CBZ-*L*-phenylalanine *N*-hydroxy-succinimide ester, *N*-α-CBZ-*L*-glutamine *N*-hydroxy-succinimide ester, *N*-α-CBZ-*L*-isoleucine *N*-hydroxy-succinimide ester, *N*-α-CBZ-*L*-alaninal *N*-hydroxy-succinimide ester, *N*-α-CBZ-*L*-glycine *N*-hydroxy-succinimide ester and *N*-α-CBZ-*L*-tryptophan *para*-nitrophenyl ester.

A fourth aspect of the invention provides a method of treating a subject in need of treatment with a transglutaminase inhibitor comprising administering to said subject a compound according to the first aspect of the invention or a pharmaceutical formulation according to the second aspect of the invention.

Preferably, the compound according to the first aspect of the invention or a pharmaceutical formulation according to the second aspect of the invention is administered in an amount sufficient to inhibit, at least in

part, tTGase-mediated protein modification (*i.e.* cross-linking). More preferably, the compound or formulation is administered in an amount sufficient to inhibit tTGase-mediated protein cross-linking by at least 10%, for example, at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95%. Most preferably, the compound or formulation is administered in an amount sufficient to inhibit completely tTGase-mediated protein cross-linking.

TGase-mediated protein modification may be measured by methods known in the art. For example, detection of the isodipeptide ε(γ-glutamyl)lysine in body fluids can be used as an indirect measure of the frequency of crosslinking in diseases which involve this protein cross link. Hence, a reduction of the isodipeptide in the body fluid provides an indirect measure of reduced protein crosslinking (see Nemes *et al.*, 2002, *Minerva Biotechnology* **14**, 183).

Alternatively, a tissue biopsy may be taken and analysed, for example by ion exchange or reversed phase HPLC after proteolytic digestion of the material (Griffin & Wilson, 1984, *Mol. Cell Biochem.* **58**:37- 49), or by staining biopsy sections and analysing by immunohistochemistry (Skill *et al.*, 2001, 81:705-716).

More preferably, the subject has a disease/disorder selected from the group consisting of fibrosis, scarring, neurodegenerative diseases (such as Huntingdon's disease and Alzheimer's disease), autoimmune diseases (such as coeliac disease), thrombosis, proliferative disorders (*i.e.* cancer), AIDS, psoriasis and chronic inflammatory diseases of the joints, including rheumatoid arthritis and osteoarthritis.

Most preferably, the fourth aspect of the invention provides a method for treating fibrosis and/or renal scarring in a subject comprising administering to said subject a compound according to the first aspect of the invention or a pharmaceutical formulation according to the second aspect of the invention in an amount sufficient to inhibit fibrosis and/or renal scarring.

By "renal scarring" we mean loss of renal architecture and cell depletion in the glomeruli and renal tubules accompanied by the increased accumulation and deposition of extracellular matrix components, such as collagen.

It will be appreciated by those skilled in the art that treatment may be prophylactic and/or therapeutic. For example, the compounds and formulations of the invention may be used to slow and/or to prevent the onset of a disease/disorder in the subject being treated. Alternatively, or in addition, the compounds and formulations of the invention may be used to reduce or eradicate the symptoms of a disease/disorder in the subject being treated.

It will be further appreciated by those skilled in the art that the compound or formulation of the first and second aspects of the invention, respectively, may be administered by any route known or developed in the art. Thus, the compound or formulation may be administered by parenteral injection (*e.g.* intravenous, subcutaneous or intramuscular), by inhalation or nasal administration, or orally.

Preferably, the compound or formulation is administered systemically, for example intravenously. Alternatively, the compound or formulation is

administered topically, *e.g.* at or near a target site where TGase-mediated protein modification is to be inhibited.

Treatment with a compound or formulation according to the invention may consist of a single dose or a plurality of doses over a period of time. Advantageously, the compound or formulation is administered repeatedly.

Compounds and formulations of the invention may also be administered by a surgically implanted device that releases the compound or formulation directly to the required site, for example in the vicinity of a solid tumour.

It will be appreciated by persons skilled in the art that a subject treated using the method according to the fourth aspect of the invention may be any mammal. Preferably, the subject is human. Alternatively, the subject is a dog, cat, horse, or other domestic or farm mammalian animal.

In a preferred embodiment of the method according to the fourth aspect of the invention, the subject has cancer. In alternative preferred embodiments, the method is for treating fibrosis and/or scarring in the subject.

A fifth aspect of the invention provides a compound according to the fourth aspect of the invention for use in medicine.

A sixth aspect of the invention provides the use of a compound according to the first aspect of the invention in the preparation of a medicament for inhibiting a transglutaminase, for example a tissue transglutaminase.

In a preferred embodiment, the medicament is for treating a disease/disorder selected from the group consisting of fibrosis, scarring (*e.g.* renal scarring or hypertrophic scarring of the skin), neurodegenerative diseases (such as Huntingdon's disease and Alzheimer's disease), autoimmune diseases (such as coeliac disease), thrombosis, proliferative disorders (*i.e.* cancer), AIDS, psoriasis and chronic inflammatory diseases of the joints, including rheumatoid arthritis and osteoarthritis.

Advantageously, the medicament is for treating (including inhibiting and/or preventing) fibrosis and/or scarring, and in particular renal scarring.

A fifth aspect of the invention provides a method for preventing or treating rejection of a transplanted organ comprising contacting the organ with a compound according to the first aspect of the invention or a formulation according to the second aspect of the invention. Thus, the invention provides the use of a compound according to the first aspect of the invention in the preparation of a medicament for preventing or treating rejection of a transplanted organ.

Preferably, the organ is a heart, lung, kidney or liver.

Most preferably, the organ is a kidney. Kidneys that are to be transplanted often show some upregulation of tissue transglutaminase and possibly other transglutaminases. Moreover, kidneys which are rejected after transplantation often exhibit excessive scarring and upregulation of transglutaminase activity and crosslinking (Abo-Zenah *et al.*, 2001, *J. Am. Soc. Nephrol.* **12**, 4454A). Such tissue

degeneration and subsequent organ rejection may be prevented by treating the kidney (or other organ) with a transglutaminase inhibitor.

It will be appreciated that the compound or formulation may be delivered before, during and/or after transplantation of the organ. Thus, in one embodiment, the organ is treated prior to transplantation, for example by perfusing and/or bathing with a solution containing a compound according to the first aspect of the invention.

In an alternative embodiment, the organ is treated during and/or after transplantation into a patient. Advantageously, the compound or formulation is delivered at or near the site of the transplant, for example by local administration.

Preferably, the medicament is for treating a proliferative disorder, especially cancers manifesting themselves as solid tumours.

Preferred, non-limiting examples which embody certain aspects of the invention will now be described, with reference to the following figures in which:

Figure 1 shows a synthesis route for the production of an exemplary compound according to the first aspect of the invention, namely *N*-Benzyloxycarbonyl-*L*-phenylalanyl-6-dimethylsulfonium-5-oxo-*L*-norleucine bromide salt ('Compound 281'). In step (i) the *N*-α-CBZ-protected amino acid *N*-hydroxysuccinimide ester is reacted with 6-diazo-5-oxo-L-norleucine (DON) to produce Z-phenylalaninyl bromomethyl ketone, which is then reacted with dimethylsulphide to produce *N*-benzyloxycarbonyl-*L*-phenylalanyl-6-dimethylsulfonium-5-oxo-*L*-norleucine bromide salt.

Reagents and conditions for each step are as follows:

(i) Triethylamine (TEA), THF, H_20;
(ii) HBr, ethyl acetate; and
(iii) Dimethyl sulphide.

Figures 2 to 10 show the effect of increasing concentrations of exemplary compounds of the invention (and prior art compound 1,3-dimethyl-2-(2-oxopropylsulfanyl)-3H-1,3-diazol-1-ium-chloride) on the inhibition of guinea pig liver transglutaminase (tTG), as measured by an enzyme-linked sorbent assay (ELSA) (see Example 2, below). The concentration of the test compound test is given in μM, along the x-axis. The compounds tested are as follows:

Figure	*Compound tested*
2	*N*-Benzyloxycarbonyl-*L*-phenylalanyl-6-dimethylsulfonium-5-oxo-*L*-norleucine bromide salt ("*Compound 281*")
3	1,3-dimethyl-2-(2-oxopropylsulfanyl)-3H-1,3-diazol-1-ium-chloride ("*Compound 283*")
4	*N*-Benzyloxycarbonyl-*L*-glutaminyl-6-dimethylsulfonium-5-oxo-*L*-norleucine bromide salt ("*Compound 285*")
5	*N*-Benzyloxycarbonyl-*L*-isoleucinal-6-dimethylsulfonium-5-oxo-*L*-norleucine bromide salt ("*Compound 286*")
6	*N*-Benzyoxycarbonyl-*L*-phenylalanyl-7-dimethyl-sulfonium-6-oxo-heptanoic acid bromide salt ("*Compound 287*")

7	N-Benzyloxycarbonyl-L-phenylalanyl-L-5-dimethylsulfonium-4-oxo-norvaline bromide salt ("*Compound 289*")
8	*N*-Benzyloxycarbonyl-*L*-alaninal-6-dimethylsulfonium-5-oxo-*L*-norleucine bromide salt ("*Compound 291*")
9	*N*-Benzyloxycarbonyl-*L*-glycinal-6-dimethylsulfonium-5-oxo-*L*-norleucine bromide salt ("*Compound 292*")
10	*N*-Benzyloxycarbonyl-*L*-tyrosinal-6-dimethylsulfonium-5-oxo-*L*-norleucine bromide salt ("*Compound 293*")

Figure 11 shows SDS-PAGE data demonstrating inhibition of tTGase-mediated crosslinking of fibronectin following treatment with exemplary compounds of the invention (see Example 3). Key: 'tTG' = tissue transglutaminase, 'degr. fragments' = degradation fragments, 'Fn' = fibronectin, 'Polymers' = cross-linked fibronectin polymers, '281' = *N*-Benzyloxycarbonyl-*L*-phenylalanyl-6-dimethylsulfonium-5-oxo-*L*-norleucine bromide salt, '285'/'Rob285' = *N*-Benzyloxycarbonyl-*L*-glutaminyl-6-dimethyl-sulfonium-5-oxo-*L*-norleucine bromide salt.

Figure 12 shows (a) representative Masson's Trichrome stained sections at 100x magnification and (b) collagen III stained sections at 200x magnification from kidneys of rats treated for 84 days with inhibitor *N*-Benzyloxycarbonyl-*L*-phenylalanyl-6-dimethylsulfonium-5-oxo-*L*-norleucine bromide salt (designated 'SNx + 281') or 1,3-dimethyl-2-(2-oxopropylsulfanyl)-3H-1,3-diazol-1-ium-chloride (designated 'SNx + 283'). SNx indicates animals in which a subtotal nephrectomy has been performed. These animals either had PBS (SNx) or TGase inhibitor

compound 281 or 283 (SNx+281 and SNX+283, respectively) instilled into their kidney. 'SNc' refers to sham operated animals and 'SNx' refers to animals which have had PBS instilled into their kidneys. Five animals per group were used (see Example 4).

Figure 13 shows Quantative Image Analysis of (a) Masson's Trichrome staining and (b) collagen III staining in the kidney sections from 90 day animals following treatment with inhibitor *N*-Benzyloxycarbonyl-*L*-phenylalanyl-6-dimethylsulfonium-5-oxo-*L*-norleucine bromide salt (designated 'SNx + 281') and 1,3-dimethyl-2-(2-oxopropylsulfanyl)-3H-1,3-diazol-1-ium-chloride (designated 'SNx + 283'). 'Snc' and 'SNx' are referred to as in legend to Figure 12 above. Five animals per group were used (see Example 4).

Figure 14 shows the inhibition of TGase activity in kidneys of rats treated with compounds having TGase inhibitor activity. Figure 14 (a) is a histogram showing semi-quantative analysis of *in situ* TGase activity in cryostat sections taken from kidneys of SNx rats treated for 28 days with the inhibitors *N*-Benzyloxycarbonyl-*L*-phenylalanyl-6-dimethyl-sulfonium-5-oxo-*L*-norleucine bromide salt (designated 'SNx + 281') and 1,3-dimethyl-2-(2-oxopropylsulfanyl)-3H-1,3-diazol-1-ium-chloride (designated 'SNx + 283'). Data show emission from Leica confocal laser microscope from TRITC-extravidin bound to TGase incorporated biotin cadaverine. 'SNc' refers to control kidneys obtained from animals on which a sham operation was performed without subtotal nephrectomy. 'SNx' refers to subtotal nephrectomy. Inhibitors were delivered to the kidney by mini-pumps (see Example 5). Figure 14 (b) is a histogram showing TGase activity measured by ^{14}C–putrescine incorporation into N, N'-dimethyl casein at day 84 in kidney homogenates of SNx rats treated with the inhibitors *N*-Benzyloxycarbonyl-*L*-phenylalanyl-6-di-methyl-

sulfonium-5-oxo-*L*-norleucine bromide salt (designated 'SNx + 281') and 1,3-dimethyl-2-(2-oxopropylsulfanyl)-3H-1,3-diazol-1-ium-chloride (designated 'SNx + 283'). Five animals per group were used (see Example 4).

Figure 15 shows the effect on renal function in rats of 84 days treatment with the inhibitors *N*-Benzyloxycarbonyl-*L*-phenylalanyl-6-dimethylsulfonium-5-oxo-*L*-norleucine bromide salt (designated 'SNx + 281') and 1,3-dimethyl-2-(2-oxopropylsulfanyl)-3H-1,3-diazol-1-ium-chloride (designated 'SNx + 283'), as determined using measurements of (a) proteinuria and (b) creatinine clearance. 'SNc' refers to control kidneys obtained from animals on which a sham operation was performed without subtotal nephrectomy. 'SNx' refers to subtotal nephrectomy. Five animals per group were used (see Example 4).

EXAMPLES

EXAMPLE 1 – SYNTHESIS OF EXEMPLARY TGASE INHIBITORS

General procedures

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1600 series FT-IR instrument. 1H spectra were recorded on a JEOL E-270 instrument at 270 Mz. 13C spectra were recorded on the same instrument at 67.8 MHz. All NMR samples were prepared in deuteriochloroform unless otherwise stated. Chemical shifts are reported relative to the internal standard tetramethylsilane and quoted as ppm.

Tetrahydrofuran (THF) was freshly distilled from sodium benzophenone ketyl before use. Ether was distilled from lithium aluminium hydride and stored over sodium wire. Methanol and ethanol were distilled and stored over 5 Å molecular sieves. *N,N*-dimethylformamide was distilled from calcium hydride and stored over 5 Å molecular sieves. Chloroform, dichloromethane and acetone were dried over granular calcium chloride. Solvents used for flash column chromatography were distilled before use.

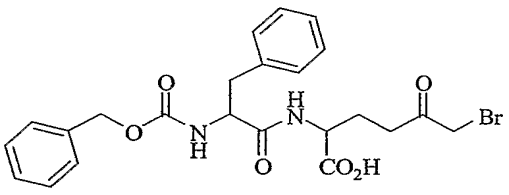
Flash chromatography was carried out using Fluka silica gel 60, 220-240 mesh size. Thin layer chromatography was carried out using Whatman silica gel 60A F254 pre-coated glass plates.

Synthesis of 6-diazo-5-oxo-L-norleucine (DON)

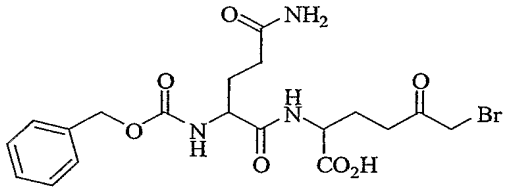
The intermediate 6-diazo-5-oxo-L-norleucine, DON, was prepared as previously described in Coutts & Saint (1998) *Tetrahedron Lett.* **39**:3243.

Synthesis of 6-bromo-5-oxo-L-norleucine derivatives

The following intermediates were synthesised:



1 (R279)



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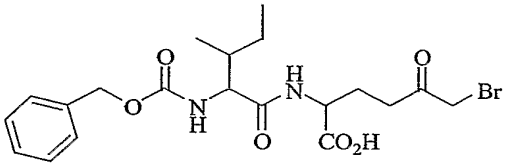

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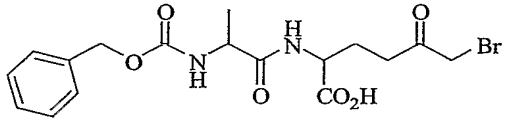


4

To an ice-cold solution of the appropriate *N*-α-CBZ-protected amino acid *N*-hydroxysuccinimide ester and DON (1 eqv.) in a 1:1 mixture of THF/water (0.5M) was added triethylamine (1.5 eqv.). The reaction mixture was stirred for 2 h. at 0°C and the solvent removed under high vacuum at room temperature. The residue was dissolved in ethyl acetate and treated with a 1:1 mixture of HBr and acetic acid dropwise until gas evolution ceased. The resulting mixture was stirred for a further 10 min. and ethyl acetate added and the organic layer washed with water (x3) brine (x1) and dried over $MgSO_4$. Removal of the solvent *in vacuo*

afforded a colourless solid which was recrystallised from an appropriate solvent to give the product in typically 70-80% yield.

(a) *N*-α-Benzyloxycarbonyl-*L*-phenylalanyl-6-bromo-5-oxo-*L*-norleucine

N-α-Benzyloxycarbonyl-*L*-phenylalanyl-6-bromo-5-oxo-*L*-norleucine (see '1' above) was prepared from DON and *N*-α-CBZ-*L*-phenylalanine *N*-hydroxysuccinimide ester (Novabiochem cat. no. 04-12-0573) (see Figure 1)

m.p. 132-133°C (ethyl acetate), (Found: C, 54.42; H, 5.14; N, 5.44. $C_{23}H_{25}BrN_2O_6$ requires C, 54.66; H, 4.99; N, 5.54%.); ν_{max} (KBr)/cm^{-1} 3294, 1719, 1689, 1655; δ_H (d_6 acetone) 1.9, 2.2 and 2.7 (4 H, m), 2.9-3.2 (2 H, m), 4.2 (2 H, s), 4.5 (2 H, m), 5.0 (2 H, s), 6.6 (1 H, d), 7.3 (10 H, ArH), 7.6 (1 H, d); δ_C (d_6 acetone) 27.0, 36.0, 36.3, 38.6, 51.8, 57.2, 66.7, 127.3, 128.5, 128.6, 129.1, 129.2, 130.2, 137.9, 153.5, 172.4, 173.7, 201.0.

(b) *N*-α-Benzyloxycarbonyl-*L*-glutaminyl-6-bromo-5-oxo-*L*-norleucine

N-α-Benzyloxycarbonyl-*L*-glutaminyl-6-bromo-5-oxo-*L*-norleucine (see '2' above) was prepared from DON and *N*-α-CBZ-*L*-glutamine *N*-hydroxysuccinimide ester (Bachem cat. no. C-1625)

m.p. 161-163°C (iso-propanol, dec.), ν_{max} (KBr)/cm^{-1} 3423, 3346, 1702, 1684, 1638; δ_H (d_4 methanol) 1.8, 1.9, 2.2 and 2.6 (8 H, m), 3.9 (2 H, s), 4.1 (1 H, m), 4.3 (1 H, m), 5.0 (2 H, s), 7.3 (5 H, ArH), 7.6 (1 H, d); δ_C (d_4 methanol) 26.8, 28.9, 32.5, 35.6, 36.5, 52.6, 55.9,

67.7, 128.8, 129.0, 129.5, 130.2, 136.9, 154.5, 171.9, 174.5, 202.6. MS: *m/z* Calcd for $C_{19}H_{24}BrN_3O_7$: 485 (M-Br = 406). Observed 406.

(c) *N*-α-Benzyloxycarbonyl-*L*-isoleucinal-6-bromo-5-oxo-*L*-norleucine

N-α-Benzyloxycarbonyl-*L*-isoleucinal-6-bromo-5-oxo-*L*-norleucine (see '3' above) was prepared from DON and *N*-α-CBZ-*L*-isoleucine *N*-hydroxysuccinimide ester (Novabiochem cat. No. 04-12-0560)

m.p. 182-184°C (ethyl acetate, dec.), ν_{max} (KBr)/cm^{-1} 3296, 1720, 1684, 1660; δ_H (d_4 methanol) 0.9, 1.2 and 1.5 (6 H, m), 1.9, 2.2 and 2.7 (4 H, m), 3,9 (1 H d), 4.0 (2 H, s), 4.4 (2 H, m), 5.1 (2 H, s), 7.3 (5 H, ArH); δ_C (d_4 methanol) 11.2, 15.9, 25.9, 26.8, 35.6, 36.4, 37.9, 52.3, 61.1, 67.6, 128.7, 129.0, 129.5, 138.1, 154.6, 174.4, 174.5, 202.8. MS: *m/z* Calcd for $C_{20}H_{27}BrN_2O_6$: 470 (M-Br = 391). Observed 473, 471, 391.

(d) *N*-α-Benzyloxycarbonyl-*L*-alaninal-6-bromo-5-oxo-*L*-norleucine

N-α-Benzyloxycarbonyl-*L*-alaninal-6-bromo-5-oxo-*L*-norleucine (see '4' above) was prepared from DON and *N*-α-CBZ-*L*-alanine *N*-hydroxysuccinimide ester (Novabiochem cat. No. 04-12-0512)

m.p. 82-85°C (DCM/ether, dec.), ν_{max} (KBr)/cm^{-1} 3294, 1719, 1686, 1660; δ_H (d_6 acetone) 1.4(3 H, d), 1.9, 2.2 and 2.6 (4 H, m), 4.1, (2 H s), 4.2 (2 H, m), 4.4 (1 H, m), 5.1 (2 H, s), 7.3 (5 H, ArH); δ_C (d_6 acetone) 26.3, 28.9, 34.5, 51.3, 51.9, 66.7, 68.6, 128.7, 129.0, 129.5,

138.1, 154.6, 173.2, 174.4, 202.6. MS: *m/z* Calcd for $C_{17}H_{21}BrN_2O_6$: 428 (M-Br = 349). Observed 429, 349.

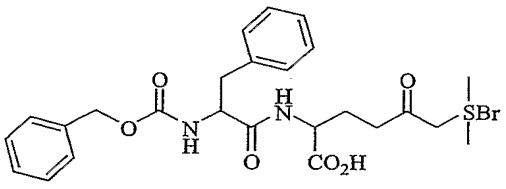
N-α-Benzyloxycarbonyl-*L*-glycinal-6-bromo-5-oxo-*L*-norleucine was prepared from DON and *N*-α-CBZ-*L*-glycine *N*-hydroxysuccinimide ester (Novabiochem cat. No. 04-12-0511) and used without further purification.

N-α-Benzyloxycarbonyl-*L*-tyrosinal-6-bromo-5-oxo-*L*-norleucine was prepared from DON and *N*-α-CBZ-*L*-tyrosine 4-nitrophenyl ester (Fluka cat. No. 97300) and used without further purification.

Preparation of amino acid derived TGase inhibitors

Sulfonium salts of the above intermediates were prepared using a modification of procedures previously reported by Pliura *et al.* (1992) *J. Enzyme Inhibition* **6**, 2768 and Shaw (1988) *Biol. Chem.*, **263**, 2768.

The bromomethyl ketone was dissolved in the minimum amount of dry methanol to achieve solution. Methyl sulfide (2.5-7.5 eqv.) was added and the solution left in a tightly stoppered flask for 24-48 h. until the reaction was judged complete by TLC. Purification was achieved by dissolving the residue in deionised water and extracting the organic soluble impurities with ethyl acetate. Freeze drying the aqueous portion afforded the product salts as colourless solids in typically 80-90% yields.



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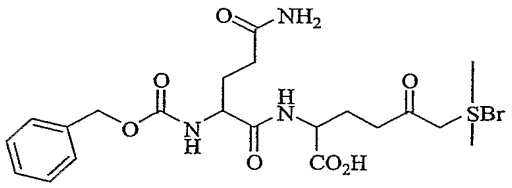

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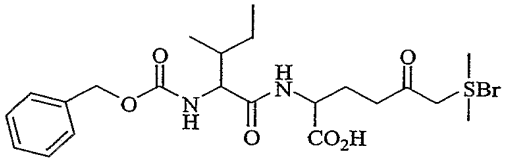

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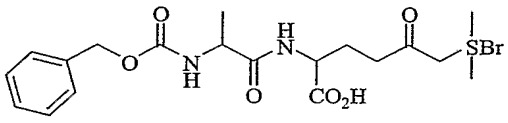

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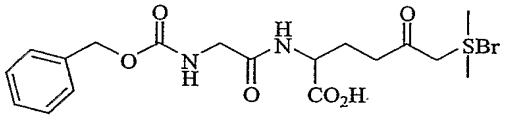

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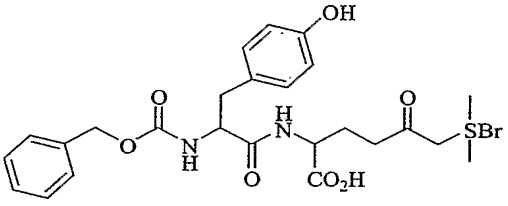


293

(a) *N*-Benzyloxycarbonyl-*L*-phenylalanyl-6-dimethylsulfonium-5-oxo-*L*-norleucine bromide salt (see '281' above)

m.p. 90-92°C, (Found: C, 52.89; H, 4.89; N, 5.05. $C_{25}H_{31}BrN_2O_6S$ requires C, 52.91; H, 5.51; N, 4.94%.); ν_{max} (KBr)/cm^{-1} 3296, 1715, 1700, 1661; δ_H (d_6 acetone) 1.9, 2.2 and 2.7 (4 H, m), 2.9-3.1 (2 H, m), 3.2 (6 H, s), 4.6 (2 H, m), 5.0 (2 H, s), 5.4 (1 H, d), 7.3 (10 H, ArH), 7.4 (1 H, d); δ_C (d_6 acetone) 25.2, 36.1, 38.5, 41.8, 51.9, 56.3, 60.6, 66.3, 128.4, 128.6, 129.0, 129.2, 130.3, 138.2, 138.6, 153.3, 168.7, 173.2, 202.0.

(b) *N*-Benzyloxycarbonyl-*L*-glutaminyl-6-dimethylsulfonium-5-oxo-*L*-norleucine bromide salt (see '285' above)

m.p. 100°C (dec.), ν_{max} (KBr)/cm^{-1} 3423, 3346, 1702, 1684, 1638; δ_H (DMSO-D_6) 1.7, 1.9, 2.1 and 2.6 (8 H, m), 2.8 (6 H, s), 3.3 (1 H, br),

3.9 (2 H, s), 4.2 (1 H, m), 4.7 (2 H, m), 5.0 (2 H, s), 6.7 (1 H, s), 7.3 (5 H, ArH), 7.4 (1 H, d), 8.2 (1 H, d); δ_C (DMSO-D_6) 24.5, 25.2, 27.7, 31.5, 37.5, 50.6, 53.5, 54.3, 65.4, 127.6, 127.8, 128.3, 128.4, 136.9, 155.9, 172.0, 172.9, 173.8, 201.4. MS: *m/z* Calcd for $C_{21}H_{30}BrN_3O_7S$: 547.09878 (M-Br = 468.18045). Observed 468.17769.

(c) *N*-Benzyloxycarbonyl-*L*-isoleucinal-6-dimethylsulfonium-5-oxo-*L*-norleucine bromide salt (see '286' above)

m.p. 111-114°C (dec.), ν_{max} (KBr)/cm^{-1} 3424, 1717, 1700, 1664; δ_H (DMSO-D_6) 0.9, 1.2 and 1.5 (6 H, m), 1.9, 2.2 and 2.7 (4 H, m), 3.2 (6 H, s), 3,9 (1 H d), 4.1 (2 H, s), 4.4 (1 H, m), 5.1 (2 H, s), 7.3 (5 H, ArH); δ_C (DMSO-D_6) 11.2, 15.9, 25.9, 26.8, 35.6, 36.4, 37.9, 52.3, 61.1, 67.6, 128.7, 129.0, 129.5, 138.1, 154.6, 174.4, 174.5, 202.8. MS: *m/z* Calcd for $C_{22}H_{33}BrN_2O_6S$: 532 (M-Br = 453). Observed 453.

(d) *N*-Benzyloxycarbonyl-*L*-alaninal-6-dimethylsulfonium-5-oxo-*L*-norleucine bromide salt (see '291' above)

m.p. 100-102 °C (dec.), ν_{max} (KBr)/cm^{-1} 3426, 1716, 1698, 1660; δ_H (d_6 DMSO) 1.2 (3 H, d), 1.8, 2.0 and 2.7 (4 H, m), 2.9 (6H, s), 4.0, (1 H, m), 4.2 (1 H, m), 4.8 (2 H, m), 5.0 (2 H, s), 7.3 (5 H, ArH); δ_C (d_6 DMSO) 24.5, 37.4, 49.8, 50.6, 52.0, 53.5, 65.3, 66.3, 127.7, 127.8, 128.3, 137.0, 155.7, 172.8, 172.9, 201.4. MS: *m/z* Calcd for $C_{19}H_{27}BrN_2O_6S$: 490 (M-Br = 411). Observed 411.

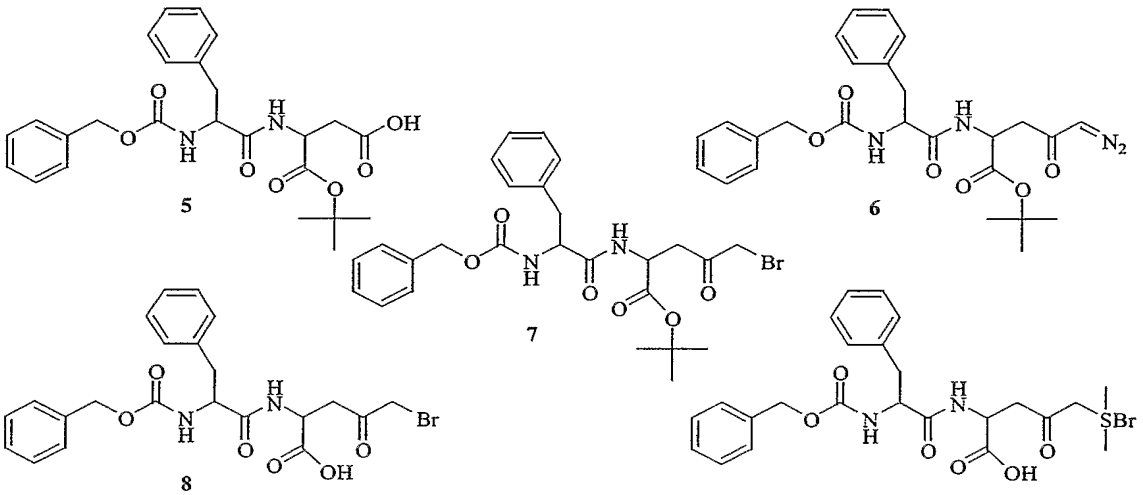
(e) *N*-Benzyloxycarbonyl-*L*-glycinal-6-dimethylsulfonium-5-oxo-*L*-norleucine bromide salt (see '292' above)

m.p. 96-99 °C (dec.), ν_{max} (KBr)/cm^{-1} 3424, 1714, 1701, 1663; δ_H (d_6 DMSO) 1.8, 2.1 and 2.6 (4 H, m), 2.9 (6H, s), 3.6, (2 H, m), 4.3 (1 H, m), 4.7 (2 H, d), 5.0 (2 H, s), 7.3 (5 H, ArH); δ_C (d_6 DMSO) 24.5, 37.4, 43.2, 50.6, 53.5, 65.4, 66.3, 127.6, 127.8, 128.3, 137.0, 156.5, 169.2, 172.9, 201.4. MS: *m/z* Calcd for $C_{18}H_{25}BrN_2O_6S$: 476 (M-Br = 397). Observed 397.

(f) *N*-Benzyloxycarbonyl-*L*-tyrosinal-6-dimethylsulfonium-5-oxo-*L*-norleucine bromide salt (see '293' above)

m.p. 111-113 °C (dec.), ν_{max} (KBr)/cm^{-1} 3426, 1716, 1700, 1666; δ_H (d_6 DMSO) 1.9 and 2.1 (2 H, m), 2.6 (4 H, m), 2.9 (6H, s), 4.2, (2 H s), 4.7 (2 H, d), 4.9 (2 H, d), 6.7 (2H, d, ArH), 7.1 (2 H, d, ArH), 7.3 (5 H, ArH); δ_C (d_6 DMSO) 24.4, 24.8, 36.5, 37.5, 50.8, 53.5, 56.3, 65.2, 114.8, 127.3, 127.7, 128.0, 128.3, 130.1, 137.0, 155.7, 172.0, 172.9, 201.4. MS: *m/z* Calcd for $C_{25}H_{31}BrN_2O_7S$: 582 (M-Br = 503). Observed 503.

Synthesis of N-Benzyloxycarbonyl-L-phenylalanyl-L-2-amino-5-dimethylsulfonium-4-oxo-norvaline bromide salt (Compound '289')



Compound '5' is made by reaction of commercially available *N*-a-CBZ-*L*-phenylalanine *N*-hydroxy-succinimide ester (Novabiochem Cat. No. 04-12-0573) and *L*-aspartic acid β-*t*-butyl ester (Novabiochem Cat. No. 04-12-5000) in water/THF (1:1) in the presence of 1.5 equivalents of triethylamine.

(a) *N*-Benzyloxycarbonyl-*L*-phenylalanyl-*L*-2-amino-5-diazo-4-oxo-norvaline *tert*-butyl ester (see '6' above)

N-Methylmorpholine (0.63 ml, 5.75 mmol) followed by *n*-butyl chloroformate (0.74 ml, 5.75 mmol) were added to a cold (-78°C) solution of *N*-α-benzyloxycarbonyl-*L*-phenylalanyl-aspartic acid ß-t-butyl ester (see '5' above) (2.35g, 5 mmol) in THF (100 ml) in an atmosphere of nitrogen. The reaction was stirred for 0.5 h. and an

ethereal solution of diazomethane, prepared from *N*-methyl-*N*-nitroso-4-toluenesulfonamide (6.23 g, 29 mmol), was added, dropwise, and the reaction left to warm to room temperature overnight. Saturated ammonium chloride solution (100 ml) was added and the mixture stirred vigorously for 5 min., then the layers were separated. Removal of the solvent *in vacuo* gave a solid residue which was recrystallised from cyclohexane/DCM to yield the product as a pale yellow solid (1.91 g, 77%).

m.p. 122-124°C, (Found: C, 62.88; H, 6.38; N, 11.01. $C_{26}H_{30}N_4O_6$ requires C, 63.15; H, 6.11; N, 11.33%.); ν_{max} (KBr)/cm^{-1} 3296, 2105, 1736, 1689, 1655; δ_H ($CDCl_3$) 1.4 (9 H, s), 2.8 and 3.2 (2 H, m), 3.6 (2 H, s), 4.4 (1 H, m), 4.6 (1 H, m), 5.1 (2 H, s), 5.2 (1 H, d), 6.9 (1 H, d), 7.3 (10H, ArH); δ_C ($CDCl_3$) 27.8, 36.2, 38.3, 49.6, 55.8, 67.2, 82.8, 127.3, 128.5, 128.6, 129.1, 129.2, 130.2, 136.0, 136.6, 153.5, 169.4, 170.7, 191.5.

(b) *N*-Benzyloxycarbonyl-*L*-phenylalanyl-*L*-2-amino-5-bromo-4-oxo-norvaline *tert*-butyl ester (see '7' above)

To a cold (0°C) solution of *N*-benzyloxycarbonyl-*L*-phenylalanyl-*L*-2-amino-5-diazo-4-oxo-norvaline *tert*-butyl ester (see '6' above) (1 g, 2 mmol) in ethyl acetate (40 ml) was added a 1:1 solution of 48% HBr/acetic acid (2 ml) dropwise. The mixture was stirred for a further 10 min. and the organic was washed with water (10 ml x3), brine (10ml) and dried over $MgSO_4$. Removal of the solvent *in vacuo* gave a solid residue which was recrystallised from cyclohexane to give a white solid (0.915 g, 84%).

m.p. 126-127°C; ν_{max} (KBr)/cm^{-1} 3294, 1738, 1690, 1654; δ_H ($CDCl_3$) 1.4 (9 H, s), 2.8 and 3.2 (2 H, m), 3.6 (2 H, s), 3.8 (2 H, s), 4.4 (1 H, m), 4.6 (1 H, m), 5.1 (2 H, s), 5.6 (1 H, d), 6.9 (1 H, d), 7.3 (10H, ArH); δ_C ($CDCl_3$) 27.8, 33.7, 38.2, 41.4, 49.3, 55.9, 82.9, 127.3, 128.5, 128.6, 129.1, 129.2, 130.2, 136.0, 136.6, 153.5, 169.4, 170.7, 199.8.

(c) *N*-Benzyloxycarbonyl-*L*-phenylalanyl-*L*-2-amino-5-bromo-4-oxo-norvaline (see '8' above)

To a solution of the *t*-butyl ester (see '7' above) (0.55 g, 1 mmol) in DCM (10 ml) was added trifluoroacetic acid (0.95 ml, 12.5 mmol) and triethylsilane (0.4 ml, 2.5 mmol). The reaction was stirred for 1.5 h. and the volatiles removed under vacuum. The resulting residue was triturated with ether to give the product as a colourless solid (0.38 g 78%),

m.p. 134-136°C, (Found: C, 53.57; H, 4.70; N, 5.65. $C_{22}H_{23}BrN_2O_6$ requires C, 53.78; H, 4.72; N, 5.70 %.); ν_{max} (KBr)/cm^{-1} 3295, 1718, 1689, 1654; δ_H ($CDCl_3$) 2.8 and 3.2 (2 H, m), 3.3 (2 H, m), 3.7 (2 H, s), 4.5 (1 H, m), 4.7 (1 H, m), 5.1 (2 H, s), 5.8 (1 H, d), 5.9 (1 H, d), 6.8 (1 H, br), 7.3 (10H, ArH); δ_C ($CDCl_3$) 33.7, 38.2, 41.4, 49.3, 55.9, 67.2, 127.0, 127.8, 128.1, 128.4, 129.3, 136.1, 136.6, 156.5, 171.4 176.7, 200.1.

(d) *N*-Benzyloxycarbonyl-*L*-phenylalanyl-*L*-2-amino-5-dimethyl-sulfonium-4-oxo-norvaline bromide salt (see '289' above)

The sulfonium salt, *N*-benzyloxycarbonyl-*L*-phenylalanyl-*L*-2-amino-5-dimethyl-sulfonium-4-oxo-norvaline bromide, was prepared as described above from bromomethyl ketone (see '8' above) (0.1 g, 0.2 mmol) and methyl sulfide (0.11 ml, 1.5 mmol). Freeze-drying afforded the product as a colourless hygroscopic solid (20 mg, 18%).

m.p. 98°C (dec.); ν_{max} (KBr)/cm^{-1} 1716, 1689, 1669; δ_H (d_4 methanol) 2.8 and 3.2 (2 H, m), 3.2 (6 H, s), 3.8 (2 H, s), 4.7 (1 H, m), 4.9 (1 H, m), 5.1 (2 H, s), 5.9 (1 H, d), 6.5 (1 H, d), 7.3 (10 H, ArH). δ_C (d_4 methanol) 25.4, 38.8, 43.9, 49.8, 57.8, 67.5, 68.1, 127.8, 128.5, 128.9, 129.5, 130.3, 136.1, 136.6, 158.3, 173.1, 174.1, 200.1.

MS: *m/z* Calc. for $C_{24}H_{29}Br\ N_2O_6S$ 552 (M-Br=473) Observed : 473

Synthesis of a higher homologue of N-Benzyloxycarbonyl-L-phenylalanyl-6-dimethylsulfonium-5-oxo-L-norleucine bromide salt (see 'Compound 288' below)

The acid was prepared from 6-diazo-*N*-(9-fluorenylmethyloxycarbonyl)-5-oxo-*L*-norleucine ethyl ester (2.53 g, 6 mmol) by the method of Coutts *et al.* Yield after flash column chromatography (ethyl acetate 100%) 1.64 g, 66%.

(a) *N*-(-9-Fluorenylmethyloxycarbonyl)-*L*-2-amino-7-diazo-6-oxo-heptanoic acid-1-ethyl ester

To a cold (0°C) solution of the acid (0.94 g, 2.3 mmol) in DCM (24 ml) was added oxalyl chloride (1.725 ml of a 2M solution in DCM, 3 3.45 mmol) dropwise. The reaction was warmed to room temperature and stirring continued for a further 40 min. The reaction was again cooled to 0°C and oxalyl chloride (1.725 ml of a 2M solution in DCM, 3.45 mmol) added dropwise. The reaction was warmed to room temperature and stirring continued for a further 2 h. The volatiles were removed under reduced pressure to give a yellow solid. The solid was dissolved in THF/acetonitrile (1:1 24 ml) and cooled in an ice bath under a blanket of nitrogen. To the solution was added trimethylsilyldiazomethane (4.6 ml of a 2M solution in hexane, 9.2 mmol) dropwise and the reaction stirred at 0°C for 11/2 h. To the mixture was added saturated ammonium chloride solution and the phases separated. The organic was washed with 10% Na_2CO_3 (5 ml, x3), brine (5 ml) and dried over Na_2SO_4. Removal of the solvent *in vacuo* gave an orange oil which was purified by flash column chromatography (3:2 petrol/ethyl acetate) to afford the product as a pale yellow solid (0.65 g, 65%).

m.p 123-124°C (CCl_4, dec.) ν_{max} (KBr)/cm^{-1} 3354, 2103, 1739, 1686, 1635; δ_H ($CDCl_3$) 1.3 (3 H, t), 1.7, 1.9 and 2.3 (6 H, m), 4.2 (3 H, m), 4.4 (3 H, m), 5.2 (1 H, s), 5.4 (1 H, d), 7.3 (4 H, m, ArH), 7.6 (2 H, ArH), 7.8 (2 H, m, ArH); δ_C ($CDCl_3$) 14.1, 20.6, 32.0, 40.1, 47.1, 53.5, 54.5, 61.6, 67.0, 120.0, 125.1, 127.0, 127.7, 141.2, 143.8, 155.9, 172.2, 192.4.

(b) *L*-2-amino-7-diazo-6-oxoheptanoic acid

The diazoketone (0.5 g, 1.15 mmol) was deprotected with piperidine as previously described to give the amino acid as a pale yellow solid (57.2 mg, 54%).

m.p. 122-124°C (Lit. m.p. 125-126°C) ν_{max} (KBr)/cm^{-1} 3436, 2108, 1630 (Weygand *et al.*, Chem. Ber. **91**, 1037-40).

(c) *N*-Benzyloxycarbonyl-*L*-phenylalanyl-7-bromo-6-oxo-heptanoic acid

The dipeptide was prepared by the method previously described to give a colourless solid (93 mg, 66%).

m.p. 108-110 °C (ethyl acetate), ν_{max} (KBr)/cm^{-1} 3296, 1715, 1700, 1661; δ_H (d_3 methanol) 1.6, 1.8 and 2.6 (6 H, m), 2.9-3.1 (2 H, m), 4.1 (2 H, s), 4.4 (2 H, m) 5.0 (2 H, s), 7.3 (10 H, ArH), 7.4 (1 H, d); δ_C (d_3 methanol) 21.0, 31.8, 39.7, 53.2, 57.7, 56.3, 67.5, 127.7, 128.6, 128.9, 129.4, 130.4, 138.1, 138.5, 158.2, 174.2, 174.8, 203.4. . MS: *m/z* Calcd for $C_{24}H_{27}BrN_2O_6$: 518 (M-Br = 439). Observed 519, 439.

(d) *N*-Benzyloxycarbonyl-L-phenylalanyl-7-dimethylsulfonium-6-oxo-heptanoic acid bromide salt (Compound '288')

The sulfonium salt was prepared as previously described, to give a colourless hygroscopic solid (48 mg, 57%).

m.p. 94-96 °C (dec.), ν_{max} (KBr)/cm^{-1} 3296, 1715, 1700, 1661; δ_H (d_6 acetone) 1.7, 1.9 and 2.7 (6 H, m), 2.8-3.1 (2 H, m), 2.9 (6 H, s), 4.4

(2 H, m), 4.9 (2 H, d), 5.0 (2 H, s), 7.3 (10 H, ArH); δ_C (d_6 acetone) 20.5, 25.3, 31.7, 39.0, 41.6, 53.1, 53.2, 57.7, 67.5, 127.7, 128.5, 128.9, 129.4, 129.5, 130.4, 138.2, 138.5, 158.2, 174.3, 174.7, 202.3. . MS: *m/z* Calcd for $C_{26}H_{33}BrN_2O_6S$: 580 (M-Br = 501). Observed 501.

EXAMPLE 2 – INHIBITION OF TGASE ACTIVITY

The efficacy of exemplary compounds of the invention in the inhibition of transglutaminase was verified by studying the dose-dependency of their effects on the activity of purified guinea pig liver transglutaminase (gplTGase), using an enzyme-linked sorbent assay (ELSA) based on biotinylated cadaverine (BTC) incorporation into *N,N'*-dimethyl casein (DMC).

Experiments were performed as follows:

Inhibition of guinea pig liver TG (TGase) was tested using an enzyme-linked sorbent assay (ELSA) based on the incorporation of biotin cadaverine (BTC) into N, N'-dimethylcasein (DMC). Microtitre plates (96-well) were coated with 100 µl of 10 mg/ml DMC in 10 mM Tris pH 7.4 overnight at 4°C. The following day, plates were washed twice with TBS-Tween pH 7.4, once with TBS pH7.4, and a reaction mix was prepared that contained 5 mM $CaCl_2$, 5 mM DTT and 0.132 mM BTC in 50 mM Tris pH 7.4. The mix was prepared so that the appropriate final concentrations would be achieved upon addition of 10 µl of 200 µg/ml TGase to 990 µl of mix to start the reaction. TGase inhibitors were initially prepared as 100 mM stock solutions in H_2O and diluted to the appropriate final concentration in the same reaction solution. Negative control samples for TGase activity consisted of mixes that did not contain BTC, and where 10 mM EDTA was substituted for 5 mM $CaCl_2$.

Following addition of TGase, 100 µl of solution was pipetted into 8 replicate wells per sample, and the reaction was allowed to proceed for 1 hour at 37°C. The reaction was terminated by removal of the solution

and the addition of 100 µl of 10 mM EDTA in PBS pH7.4. Plates were again washed twice with TBS-Tween pH 7.4, once with TBS pH 7.4, and blocked by incubation with 100 µl per well of 3%(w/v) bovine serum albumin (BSA) in PBS pH7.4 for 1 hour at room temperature. Incorporated BTC was detected by incubation with 100 µl per well of Extravidin peroxidase (EXAP) solution, diluted 1 in 5000 in blocking buffer for 1 hour at 37°C. Plates were washed as before and prior to development, plates were preincubated for 5 minutes in 0.05M phosphate-citrate buffer pH5.0 containing 0.014% (v/v) H_2O_2. The solution was removed and replaced with 100 µl per well of the same buffer containing 75 µg/ml tetramethylbenzidine (TMB). The developing reaction was allowed to proceed at room temperature for 5-15 minutes and was terminated by the addition of 50 µl of 1N H_2SO_4. The absorbance of the resulting colour was measured on a microtitre plate reader at 450 nm.

The data shown indicate a representative experiment using eight replicate samples. The mean absorbance 450nm ± SD is shown.

The effect of exemplary compounds of the invention (and control compounds) on tissue transglutaminase activity *in vitro* are shown in Figures 2 to 10.

EXAMPLE 3 – INHIBITION OF TGASE-MEDIATED PROTEIN CROSS-LINKING

Assay method

1. Preactivate TGase in 3mM DTT (where applicable) on ice for 1hr.
2. Crosslink TGase with fibronectin in 40Mm Tris/100mM NaCl at 50 µg/ml final concentration each, according to Table 1 below. Include non-activated Tgase/ preactivated Tgase controls in the presence and absence of the inhibitors to investigate potential homodimer formation.
3. Incubate at 37 °C for 2hr to allow crosslink formation to take place.
4. Solubilise crosslink in 2x Laemmli buffer. Vortex and spin down insoluble material.
5. Load 20 µg of total protein (Tgase+ Fn) on a 7% acrylamide SDS PAGE gel. Run gel at 100 mV until dye escapes from the bottom of the gel.
6. Stain with coomassie brilliant blue for 1 hr at RT.
7. De-stain in 30% methanol/10% acetic acid at RT.

TABLE 1

Component	*Control 1* Non-activated TGase	*Control 2* Pre-activated TGase	*Control 3* Pre-activated TGase + Compound 285	*Control 4* Fibronectin	*Control 5* TGase + Fibronectin	TGase + Fibronectin + Compound 281	TGase + Fibronectin + Compound 283	TGase + Fibronectin + Compound 285
Tris pH 7.4 (mM)	40	40	40	40	40	40	40	40
NaCl (mM)	100	100	100	100	100	100	100	100
TGase (µg/ml)	50	50	50	50	50	50	50	50
Fibronectin (µg/ml)		50		50	50	50	50	50
Compound 281 (µM)						250		
Compound 283 (µM)							250	
Compound 285 (µM)			250					250

Compound 281 = *N*-Benzyloxycarbonyl-L-phenylalanyl-6-dimethyl-sulfonium-5-oxo-L-norleucine bromide salt

Compound 283 = 1,3-dimethyl-2-(2-oxopropylsulfanyl)-3H-1,3-diazol-1-ium-chloride (as disclosed in US 4,968,713)

Compound 285 = *N*-Benzyloxycarbonyl-*L*-glutaminyl-6-dimethylsulfonium-5-oxo-*L*-norleucine bromide salt

SDS-PAGE data showing tTGase-mediated crosslinking of fibronectin following treatment with exemplary compounds of the invention is shown in Figure 11.

EXAMPLE 4 – INHIBITION OF KIDNEY FIBROSIS IN RATS

Method for inhibitor delivery using osmotic minipumps

Male Wistar rat of approximately 300g weight was anaesthetised using 5% halothane and maintained at 3% for the duration of the surgical procedure. The rat was subjected to a 5/6th subtotal nephrectomy (SNx) by ligation of the left renal artery and vein followed by complete nephrectomy of the left kidney. The right kidney had both the upper and lower poles ligated followed by excision of both poles. A 9-cm cannula (0.32mm bore) was sealed on one end and fenestrated between 3 and 12 mm from the sealed end. This was then inserted through the parenchyma (cut to cut / pole to pole) of the kidney so that the blunt end was just visible through one of the cut ends. This was then sealed in position using tissue glue on both ends of the kidney such that the fenestrated area was within the remnant kidney. The cannula was passed through the muscle wall, which was then stitched using reabsorbable sutures. The cannula was then attached to the regulator of a 2 ml osmotic minipump (Azlet osmotic minipump (2ml4), Charles Rivers, UK) that was loaded (primed for 15 hrs at RT) with either PBS (SNx) or TGase inhibitor 281 or 283 (SNx+281 and SNx+283, respectively) at a concentration of 50 mM (delivery 1.5 µl per hour). The pump was then positioned subcutaneously on the right upper flank of the animal and the skin sutured. The animal was then switched onto oxygen and allowed to partially regain consciousness before being returned to the cage. The pump was changed every 28 days under halothane anaesthesia. After 83 days, the animal was placed in a metabolic cage to collect a 24-hour urine sample. The animal was then anaesthetised, the remnant kidney recovered and a terminal blood sample collected.

Tissue samples were sectioned and then underwent Masson's Trichrome staining (Johnson *et al.*, 1997, **99**:2950-2960) or collagen III staining.

For collagen III staining, paraffin embedded sections (4μm) were first dewaxed and hydrated by standard protocol (xylene 10 min, 100% ethanol 5 min, 90% ethanol 5 min,75% ethanol 5 min, 50% ethanol 5 min, water 10 min) washed in PBS for 10 min and any endogenous peroxides quenched by treatment with 3% H2O2 in methanol for 10 min. After washing in PBS for 10 min sections were treated with the epitope revealing agent TUF (ID Labs Inc. Cat no BP1122) on a water bath at 92 C for 10 min then allowed to cool to room temperature. Sections were washed with PBS for 10 min and then trypsin (Zymed Labs Cat No 00-3008) digested (trypsin diluted 1:3) for 10 min at 37 C followed by two washes in PBS for 5 min each. Sections were then blocked in goat serum (Vector Labs Cat No S1000) incubated at 37 C for 30 min. The primary collagen III antibody (Goat anti-human type III collagen, Soutern Biotech Assocs diluted 1 in 10 in 0.1% bovine serum albumin [BSA] in PBS) is then added and incubated overnight at 4 C in a humidity chamber. The samples are then washed twice with 0.1% Nonidet in PBS for 5 min followed by two washes in PBS for 5 min. The secondary antibody (rabbit anti goat which is biotinylated from DAKO Cat No E0466) diluted 1 in 400 in 0.1%BSA/PBS is then added and incubated for 30 min at 37 C. The sections are then washed twice in 0.1% Nonidet in PBS and the sections then incubated with the Avidin Biotin Enzyme complex (ABC) kit (Vector Labs Cat No PK-6102) according to the manufacturers instructions for 30min at T 37^0 C. The samples are then washed twice in PBS and the reagent substrate, 3-amino-9-ethyl carbozole (AEC [Vector Labs Cat No SK4200]) added to allow colour development (approx 5-

30min). After washing twice with water for 5 min and then twice with PBS for 5 min the samples are counterstained with haematoxylin (diluted 1 in 10 from Thermo Shandon, Gill-2 haematoxylin Cat No 6765007) for 5 min, washed twice with water for 5 min, washed with PBS once and then sections mounted using glycergel prior to viewing under a light microscope.

Figure 12 shows (a) representative Masson's Trichrome stained sections and (b) collagen III stained sections from kidneys of animals in which inhibitor compound 281 (designated 'SNx + 281') and compound 283 (designated 'SNx + 283') were instilled (see Johnson *et al.*, 1999, *J. Am. Soc. Nephrol.* 10:2146-2157 for method used to induce subtotal nephrectomy).

Figure 13 shows quantative image analysis of (a) Masson's Trichrome stain and (b) collagen III stain in kidney sections from 90 day animals following treatment with inhibitor compounds 281 (designated 'SNx + 281') and 283 (designated 'SNx + 283'). Snc and SNx are referred to as above. For Masson's Trichrome staining, analysis was performed by systematically acquiring adjacent overlapping cortical fields at 100 x magnification such that 5 fields encompassed more than 80% of the cortex. Each field was then subject to 3 phase analysis using image analysis and the area of blue (collagen), red (cytoplasm) and white (lumen) determined ensuring greater than 95% coverage. The scarring index was determined by expressing the blue phase as a fraction of the cytoplasmic. Five animals per group were used and data expressed a mean values +/- S.E.M. The composite diagram showing staining in Figure 12(a) shows 1 field from each animal. For Collagen III staining the relative amounts of collagen III present (stained brown) were

determined by systematically acquiring data from 10 overlapping cortical fields at 200x magnification and expressed as Mean values ± SEM.

In situ TGase activity in kidney cryostat sections

Rat kidneys treated *in vivo* with TGase inhibitors were snap-frozen in liquid nitrogen and 14 µm sections were cut using a cryostat and allowed to air-dry. Sections were rehydrated for 10 minutes at room temperature in a solution of 5% (w/v) rabbit serum, 10 mM EDTA, 0.01% (v/v) Triton X-100 in 50 mM Tris pH7.4, containing EXAP (diluted 1 in 200) to block endogenous biotin. Following rehydration, slides were washed twice in PBS pH7.4, and sections were incubated for 1 hour at 37°C with a reaction mix containing 5 mM CaCl2, 5 mM DTT and 0.5 mM BTC in 50 mM Tris pH 7.4. Negative controls consisted of mixes that did not contain BTC, and where 10 mM EDTA was substituted for 5 mM CaCl2. A positive control was also included that contained 20 µg/ml TGase. Following incubation, slides were washed once in PBS pH 7.4 containing 10 mM EDTA, fixed in ice-cold acetone for 5 minutes and allowed to air-dry. Dried sections were blocked in 3%(w/v) BSA in PBS pH7.4 overnight at 4°C, and incorporated BTC was revealed by incubation with Streptavidin-Cy5, diluted 1 in 100 in the same buffer for 2 hours at 37°C. Slides were viewed on a Leica TCSNT confocal microscope equipped with excitation and emission filters for Cy5, and emitted fluorescence was quantified with the software supplied by the manufacturer. Figure 14a shows semi-quantitative analysis of the emission from Leica confocal laser microscope from TRITC-extravidin bound to TGase incorporated biotin cadaverine in cryostat sections taken from kidneys of SNx rats treated for 28 days with the inhibitors 281 and 283. SNc refers to control kidneys obtained from animals undergoing a sham operation with subtotal

nephrectomy. SNx refers to subtotal nephrectomy. Inhibitors were delivered to the kidney by mini pumps as outlined above. Data are mean values +/- SEM taken from 5 separate kidneys.

Analysis of 14C putrescine incorporation

A second method of assaying TGase activity, 14C putrescine incorporation into N,N'-dimethylcasein using tissue homogenates of kidneys from SNx rats treated with the inhibitors for 84 days, confirmed the effect of treatment with compounds 281 and 283 on Tgase activity (see Figure 14b).

Putrescine incorporation experiments were performed as described in Skill *et al.*, 2001, *Lab. Invest* **81**:705-716 and Lorand *et al.*, 1972, *Anal Biochem* **50**:623-631

Analysis of proteinurea, creatinine clearance, serum creatinine, urine creatinine and urine urea

Table 3 shows levels of proteinurea, creatinine clearance, serum creatinine, urine creatinine and urine urea in 90 day SNx rats in which inhibitor compounds 283 and 281 were instilled into the kidneys.

TABLE 3

	Proteinuria (mg/24 h)		**Creatinine clearance** (ml/min)		**Serum creatinine** (mM/L)	
Experiment Group	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
Control (SNc)	129	14	1.72	0.18	46	0.6
SNx	672	140	0.44	0.2	224	36
SNx + 283	835	93	0.86	0.05	114	16
SNx + 281	503	63	0.94	0.09	208	106
	Urine creatinine (mM/L)		**Serum urea** (mM/L)		**Urine urea** (mM/L)	
Experiment Group	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
Control (SNc)	12399	2538	6.22	1.1	1160	226
SNx	2551	695	31.4	4.6	244	29
SNx + 283	3138	185	16.3	2.2	272	20
SNx + 281	3999	560	22.32	4.5	372	50

Proteinuria, creatinine clearance, serum clearance, urine creatinine and urine urea were carried out by standard clinical chemistry techniques (Johnson *et al.*, 1997, *J. Clin. Invest.* **99**:2950-2960). Creatinine and urea were measured by the standard autoanalyser technique and proteinura by the Biuret method (Johnson *et al.*, *supra*). Data represent mean values ± SE, taken from 5 animals per group.

Proteinuria and creatinine clearance data are shown in histogram form in Figure15 (a) and (b), respectively.

EXAMPLE 5 - EXEMPLARY PHARMACEUTICAL FORMULATIONS

The following examples illustrate pharmaceutical formulations according to the invention in which the active ingredient is a compound of the invention.

Example A: Tablet

Active ingredient	100 mg
Lactose	200 mg
Starch	50 mg
Polyvinylpyrrolidone	5 mg
Magnesium stearate	4 mg
	359 mg

Tablets are prepared from the foregoing ingredients by wet granulation followed by compression.

Example B: Ophthalmic Solution

Active ingredient	0.5 g
Sodium chloride, analytical grade	0.9 g
Thiomersal	0.001 g
Purified water to	100 ml
pH adjusted to	7.5

Example C: Tablet Formulations

The following formulations A and B are prepared by wet granulation of the ingredients with a solution of povidone, followed by addition of magnesium stearate and compression.

Formulation A

	mg/tablet	mg/tablet
Active ingredient	250	250
Lactose B.P.	210	26
Povidone B.P.	15	9
Sodium Starch Glycolate	20	12
Magnesium Stearate	5	3
	500	300

Formulation B

	mg/tablet	mg/tablet
Active ingredient	250	250
Lactose	150	-
Avicel PH 101®	60	26
Povidone B.P.	15	9
Sodium Starch Glycolate	20	12
Magnesium Stearate	5	3
	500	300

Formulation C

	mg/tablet
Active ingredient	100
Lactose	200
Starch	50
Povidone	5
Magnesium stearate	4
	359

The following formulations, D and E, are prepared by direct compression of the admixed ingredients. The lactose used in formulation E is of the direction compression type.

Formulation D

	mg/capsule
Active Ingredient	250
Pre-gelatinised Starch NF15	150
	400

Formulation E

	mg/capsule
Active Ingredient	250
Lactose	150
Avicel ®	100
	500

Formulation F (Controlled Release Formulation)

The formulation is prepared by wet granulation of the ingredients (below) with a solution of povidone followed by the addition of magnesium stearate and compression.

	mg/tablet
Active Ingredient	500
Hydroxypropylmethylcellulose (Methocel K4M Premium) ®	112
Lactose B.P.	53
Povidone B.P.C.	28
Magnesium Stearate	7
	700

Drug release takes place over a period of about 6-8 hours and was complete after 12 hours.

Example D: Capsule Formulations

Formulation A

A capsule formulation is prepared by admixing the ingredients of Formulation D in Example C above and filling into a two-part hard gelatin capsule. Formulation B (*infra*) is prepared in a similar manner.

Formulation B

	mg/capsule
(a) Active ingredient	250
(b) Lactose B.P.	143
(c) Sodium Starch Glycolate	25
(d) Magnesium Stearate	2
	420

Formulation C

	mg/capsule
(a) Active ingredient	250
(b) Macrogol 4000 BP	350
	600

Capsules are prepared by melting the Macrogel 4000 BP, dispersing the active ingredient in the melt and filling the melt into a two-part hard gelatin capsule.

Formulation D

	mg/capsule
Active ingredient	250
Lecithin	100
Arachis Oil	100
	450

Capsules are prepared by dispersing the active ingredient in the lecithin and arachis oil and filling the dispersion into soft, elastic gelatin capsules.

Formulation E (Controlled Release Capsule)

The following controlled release capsule formulation is prepared by extruding ingredients a, b, and c using an extruder, followed by spheronisation of the extrudate and drying. The dried pellets are then coated with release-controlling membrane (d) and filled into a two-piece, hard gelatin capsule.

	mg/capsule
(a) Active ingredient	250
(b) Microcrystalline Cellulose	125
(c) Lactose BP	125
(d) Ethyl Cellulose	13
	513

Example E: Injectable Formulation

Active ingredient	0.200 g

Sterile, pyrogen free phosphate buffer (pH7.0) to 10 ml

The active ingredient is dissolved in most of the phosphate buffer (35-40°C), then made up to volume and filtered through a sterile micropore filter into a sterile 10 ml amber glass vial (type 1) and sealed with sterile closures and overseals.

Example F: Intramuscular injection

Active ingredient	0.20 g
Benzyl Alcohol	0.10 g
Glucofurol 75®	1.45 g

Water for Injection q.s. to 3.00 ml

The active ingredient is dissolved in the glycofurol. The benzyl alcohol is then added and dissolved, and water added to 3 ml. The mixture is then filtered through a sterile micropore filter and sealed in sterile 3 ml glass vials (type 1).

Example G: Syrup Suspension

Active ingredient	0.2500 g
Sorbitol Solution	1.5000 g
Glycerol	2.0000 g
Dispersible Cellulose	0.0750 g
Sodium Benzoate	0.0050 g
Flavour, Peach 17.42.3169	0.0125 ml
Purified Water q.s. to	5.0000 ml

The sodium benzoate is dissolved in a portion of the purified water and the sorbitol solution added. The active ingredient is added and dispersed. In the glycerol is dispersed the thickener (dispersible cellulose). The two dispersions are mixed and made up to the required volume with the purified water. Further thickening is achieved as required by extra shearing of the suspension.

Example H: Suppository

	mg/suppository
Active ingredient (63 μm)	250
Hard Fat, BP (Witepsol H15 - Dynamit Nobel)	1770
	2020

One fifth of the Witepsol H15 is melted in a steam-jacketed pan at 45□C maximum. The active ingredient is sifted through a 200 μm sieve and added to the molten base with mixing, using a silverson fitted with a cutting head, until a smooth dispersion is achieved. Maintaining the mixture at

45°C, the remaining Witepsol H15 is added to the suspension and stirred to ensure a homogenous mix. The entire suspension is passed through a 250 μm stainless steel screen and, with continuous stirring, is allowed to cool to 40°C. At a temperature of 38°C to 40°C 2.02 g of the mixture is filled into suitable plastic moulds. The suppositories are allowed to cool to room temperature.

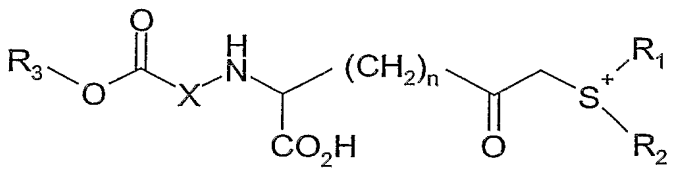
Example I: Pessaries

	mg/pessary
Active ingredient	250
Anhydrate Dextrose	380
Potato Starch	363
Magnesium Stearate	7
	1000

The above ingredients are mixed directly and pessaries prepared by direct compression of the resulting mixture.

CLAIMS

1. A compound having the following formula I:



I

wherein:

'X' represents an amino acid group;

'n' is an integer between 1 and 4;

'R_1' and 'R_2' each independently represent lower alkyl; and

'R_3' represents benzyl, t-butyl or 9-fluorenylmethyl.

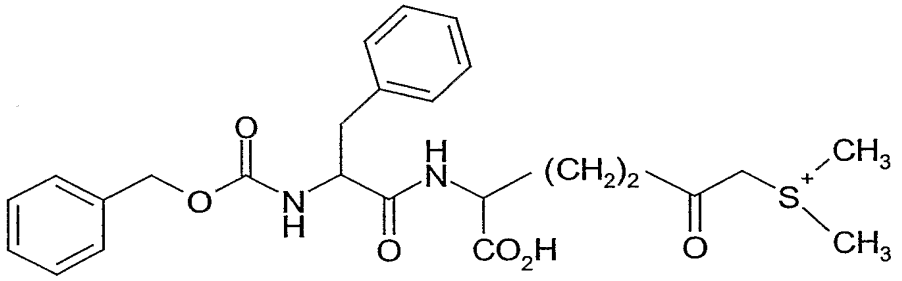
2. A compound according to Claim 1 wherein X is selected from the group consisting of phenylalanine, glutamine, isoleucine, alanine, glycine, tyrosine, proline, serine and glutamic acid.

3. A compound according to Claim 1 or 2 wherein 'n' is 2.

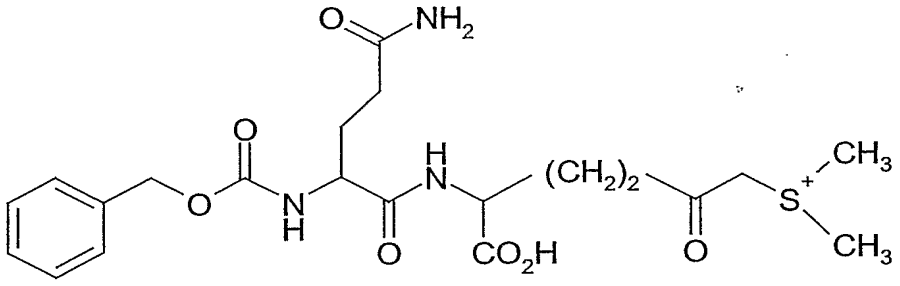
4. A compound according to any one of the preceding claims wherein 'R_1' and 'R_2' are both $-CH_3$ or $-CHCH_2$.

5. A compound according to any one of the preceding claims wherein 'R_3' is benzyl.

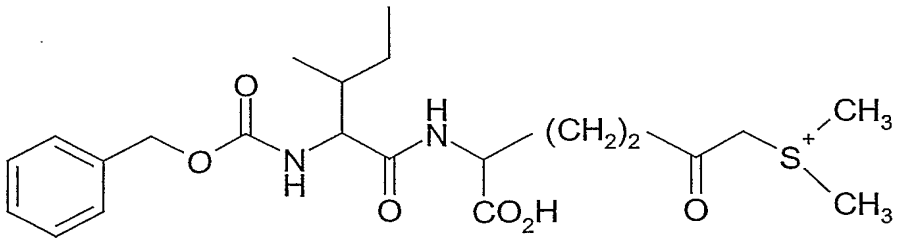
6. A compound according to any one of the preceding claims having the following formula:



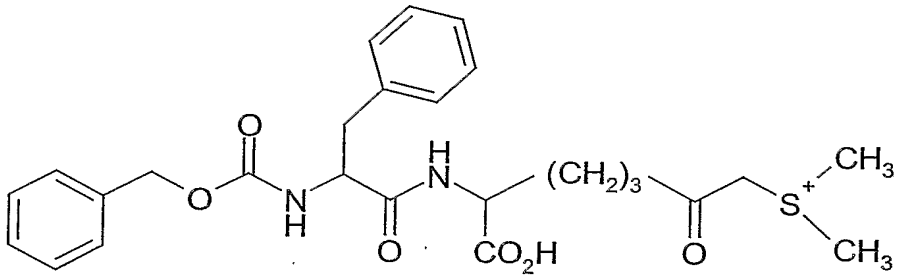
7. A compound according to any one of the preceding claims having the following formula:



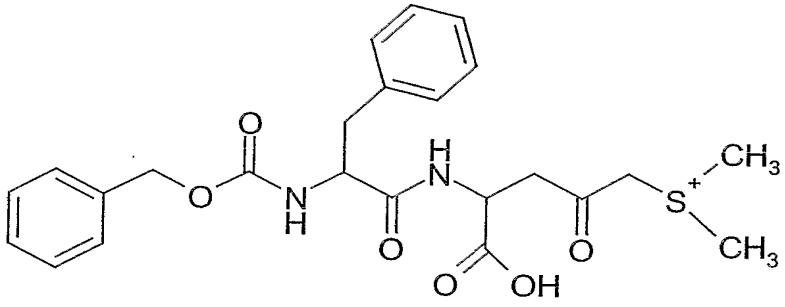
8. A compound according to any one of the preceding claims having the following formula:



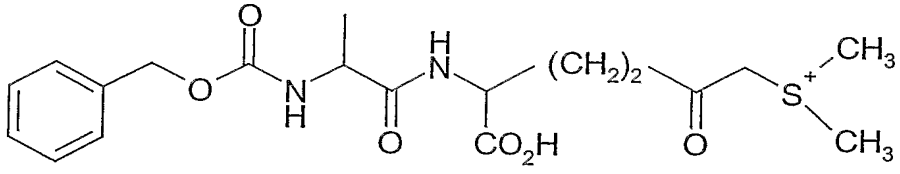
9. A compound according to any one of the preceding claims having the following formula:



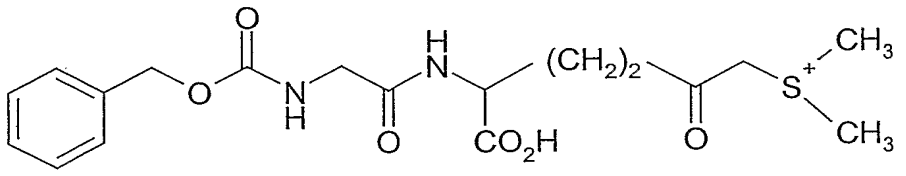
10. A compound according to any one of the preceding claims having the following formula:



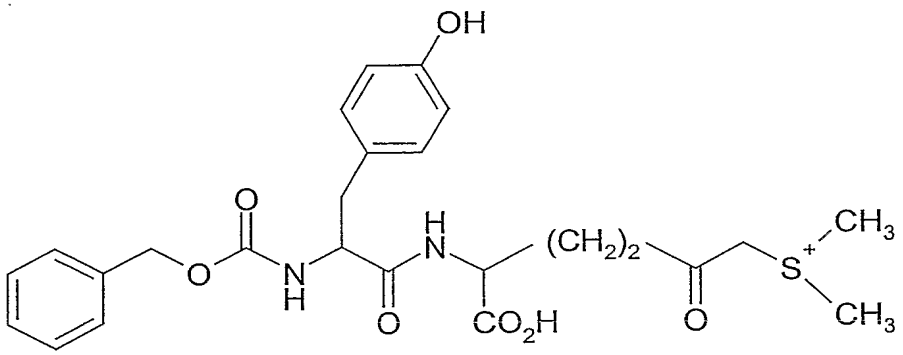
11. A compound according to any one of the preceding claims having the following formula:



12. A compound according to any one of the preceding claims having the following formula:



13. A compound having the following formula:



14. A pharmaceutical formulation comprising a compound according to any one of Claims 1 to 13 and a pharmaceutically acceptable carrier.

15. A method for making a compound according to any one of Claims 1 to 13 comprising the following steps:

(a) reacting an *N*-α-CBZ-protected amino acid *N*-hydroxy-succinimide ester or *para*-nitrophenyl ester with 6-diazo-5-oxo-L-norleucine; and

(b) reacting the bromomethyl ketone produced in step (a) with dimethyl sulphide

16. A method according to Claim 15 wherein step (a) comprises reacting an *N*-α-CBZ-protected amino acid *N*-hydroxy-succinimide or *para*-nitrophenyl ester with 6-diazo-5-oxo-L-norleucine in the presence of tetrahydrofuran (THF), water and triethylamine followed by reacting the products thereof with hydrogen bromide in the presence of ethyl acetate.

17. A method according to Claim 15 or 16 wherein the *N*-α-CBZ-protected amino acid *N*-hydroxy-succinimide ester is selected from the group consisting of *N*-α-CBZ-*L*-phenylalanine *N*-hydroxy-succinimide ester, *N*-α-CBZ-*L*-glutamine *N*-hydroxy-succinimide ester, *N*-α-CBZ-*L*-isoleucine *N*-hydroxy-succinimide ester, *N*-α-CBZ-*L*-alaninal *N*-hydroxy-succinimide ester, *N*-α-CBZ-*L*-glycine *N*-hydroxysuccinimide ester and *N*-α-CBZ-*L*-tryptophan *para*-nitrophenyl ester.

18. A method of treating a subject in need of treatment with a transglutaminase inhibitor comprising administering to said subject a compound according to any one of Claims 1 to 13 or a pharmaceutical formulation according to Claim 14.

19. A method according to Claim 18 wherein the compound or formulation is administered in an amount sufficient to inhibit, at least in part, tTGase-mediated protein modification.

20. A method according to Claim 18 or 19 wherein the subject has a disease/disorder selected from the group consisting of fibrosis, scarring, neurodegenerative diseases, autoimmune diseases, thrombosis, proliferative disorders, AIDS, psoriasis and chronic inflammatory diseases of the joints.

21. A method according to any one of Claims 18 to 20 wherein the method is for treating fibrosis and/or renal scarring.

22. A method according to Claim 20 wherein the subject has cancer.

23. A method according to Claim 20 wherein the subject has fibrosis.

24. A method according to Claim 20 wherein the subject has renal and/or tissue scarring.

25. A method according to any one of Claims 18 to 24 wherein the subject is human.

26. A method according to any one of Claims 18 to 25 wherein the compound or formulation is administered repeatedly.

27. A method according to any one of Claims 18 to 25 wherein compound or formulation is administered systemically.

28. A method according to any one of Claims 18 to 27 wherein compound or formulation is administered at or near a site of TGase-mediated protein modification.

29. A compound according to any one of Claims 1 to 13 for use in medicine.

30. Use of a compound according to any one of Claims 1 to 13 in the preparation of a medicament for inhibiting a transglutaminase

31. The use according to Claim 30 wherein the transglutaminase is a tissue transglutaminase.

32. The use according to Claim 30 wherein the medicament is for treating a disease/disorder selected from the group consisting of fibrosis, scarring, neurodegenerative diseases, autoimmune diseases, thrombosis, proliferative disorders, AIDS, psoriasis and chronic inflammatory diseases of the joints.

33. The use according to Claim 30 wherein the medicament is for treating cancer.

34. The use according to Claim 30 wherein the medicament is for treating fibrosis and/or scarring.

35. The use according to Claim 34 wherein the medicament is for treating renal scarring.

36. A method for preventing or treating rejection of a transplanted organ comprising contacting the organ with a compound according to any one of Claims 1 to 13.

37. Use of a compound according to any one of Claims 1 to 13 in the preparation of a medicament for preventing or treating rejection of a transplanted organ.

38. A method according to Claim 36 or the use according to Claim 37 wherein the organ is a heart, lung, kidney or liver.

39. A method or use according to any one of Claims 36 to 38 wherein the organ is treated prior to transplantation.

40. A method or use according to any one of Claims 36 to 38 wherein the organ is treated during and/or after transplantation into a patient.

41. A compound substantially as described herein with reference to Example 1.

42. A pharmaceutical formulation substantially as described herein with reference to Examples 1 and 6.

43. A method of treating a subject in need of treatment with a transglutaminase substantially as described herein with reference to the description.

44. Use of a compound according to any one of Claims 1 to 13 substantially as described herein with reference to the description.

45. A method for preventing or treating rejection of a transplanted organ substantially as described herein with reference to the description.

ABSTRACT

Novel compounds and methods of using the same

The present invention relates to novel compounds of Formula I wherein 'X' represents an amino acid group, 'n' is an integer between 1 and 4, 'R_1' and 'R_2' each independently represent a lower alkyl, which compounds exhibit tissue transglutaminase inhibitor activity, and R_3 represents benzyl, t-butyl or 9-Fluorenylmethyl. The present invention further relates to pharmaceutical formulations of said compound and the use thereof in the preparation of a medicament for inhibiting diseases in which transglutaminase has been implicated.. Advantageously, the medicament is for treating fibrosis, scarring and/or cancer. Additionally, the invention relates to a method of inhibiting autoimmune diseases such as coeliac disease, neurodegeneration and chronic inflammatory diseases of the joints including rheumatoid arthritis and osteoarthritis in a subject. The invention further relates to a method for preventing or treating rejection of a transplanted organ.

Figure 2

FIGURE 1

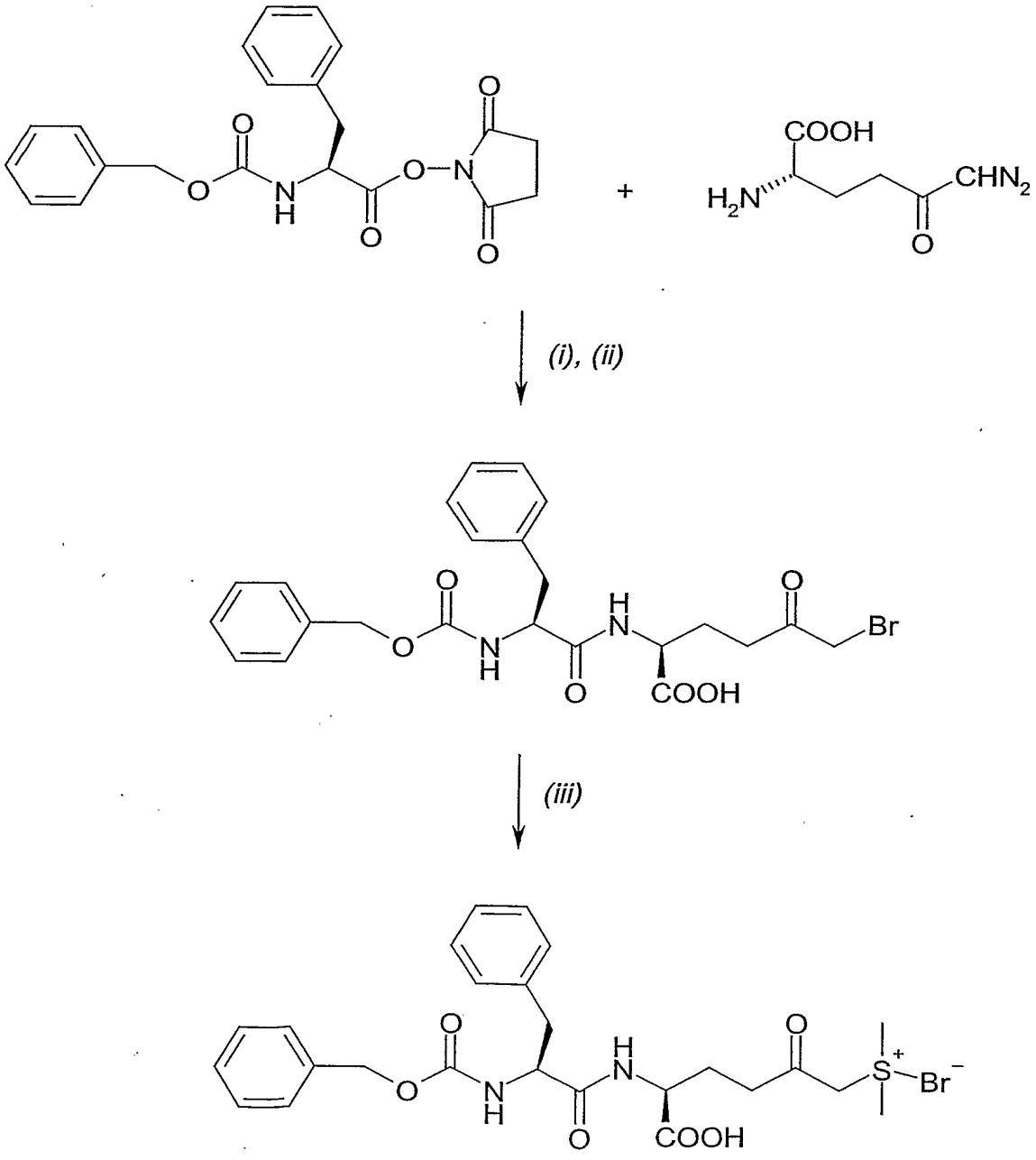


FIGURE 2

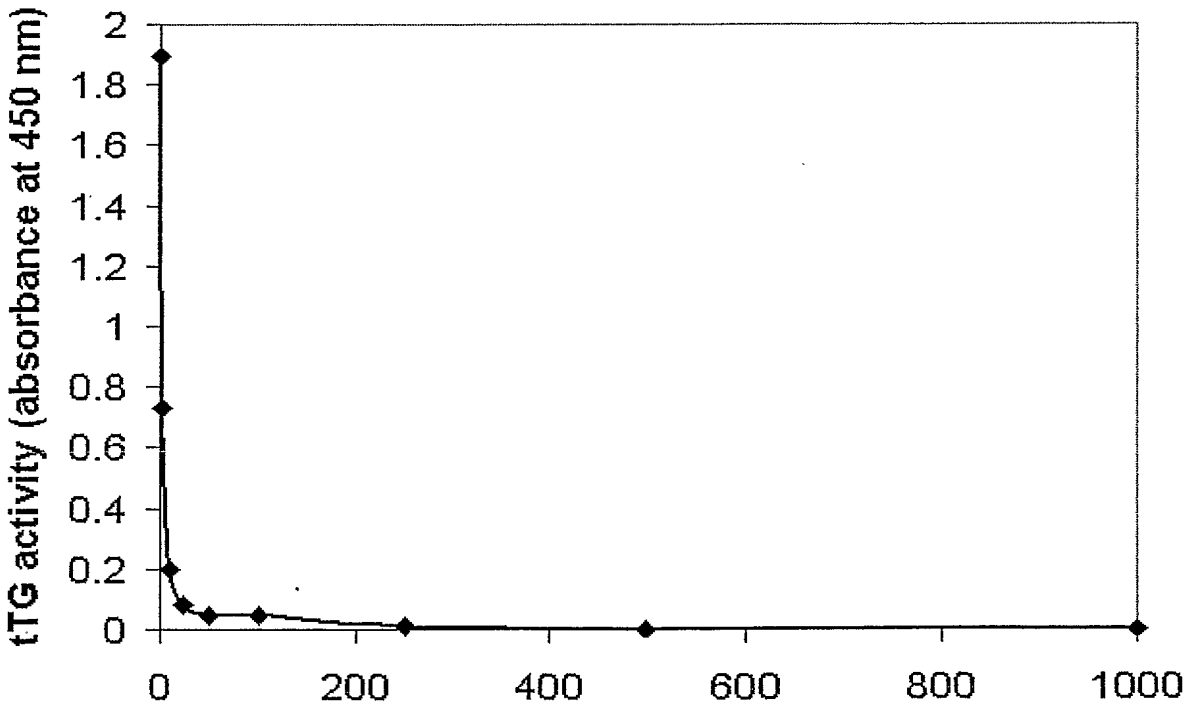

tTG activity (absorbance at 450 nm)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
200
400
600
800
1000

FIGURE 3

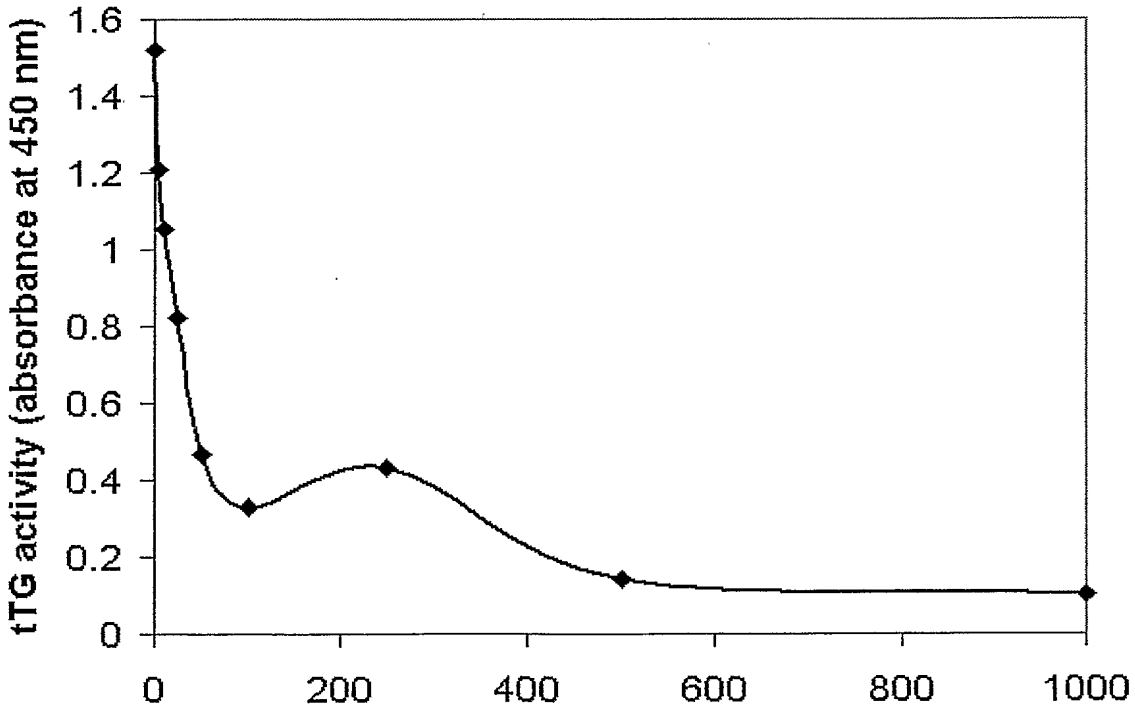

tTG activity (absorbance at 450 nm)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
200
400
600
800
1000

FIGURE 4

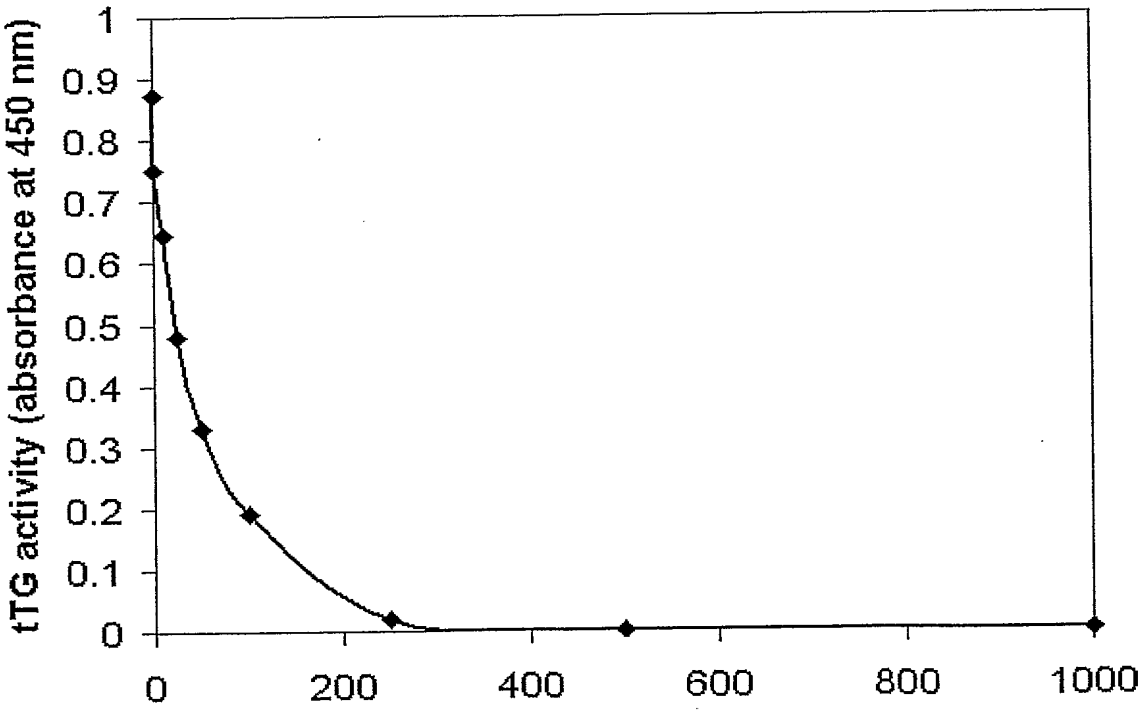

tTG activity (absorbance at 450 nm)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
200
400
600
800
1000

FIGURE 5

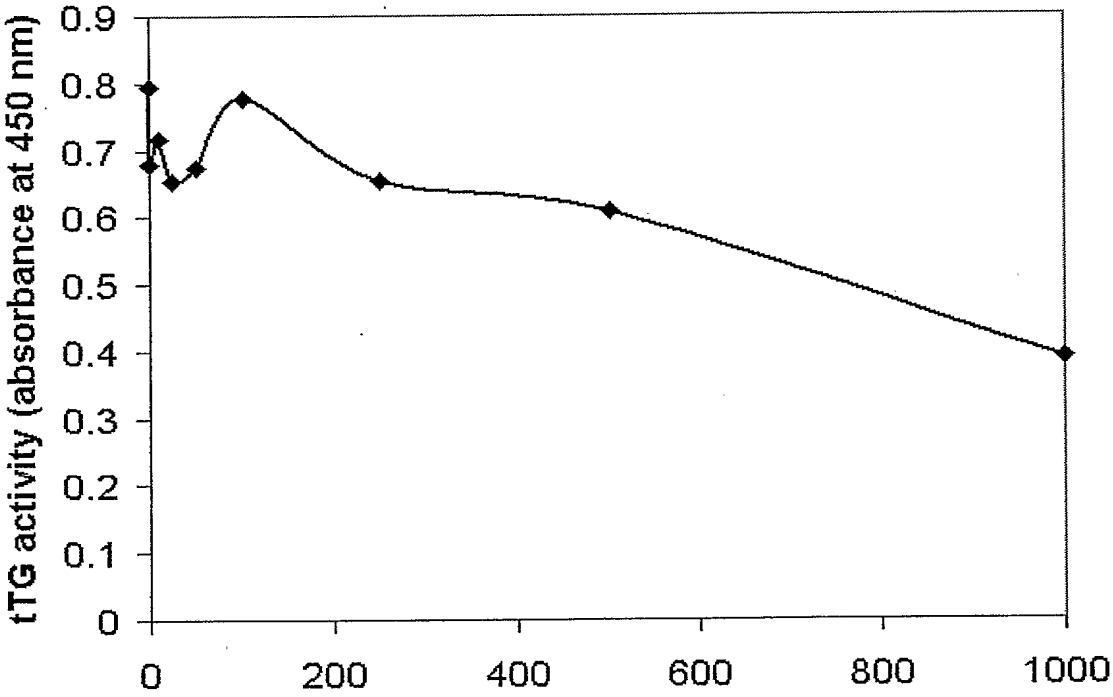

tTG activity (absorbance at 450 nm)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
200
400
600
800
1000

FIGURE 6

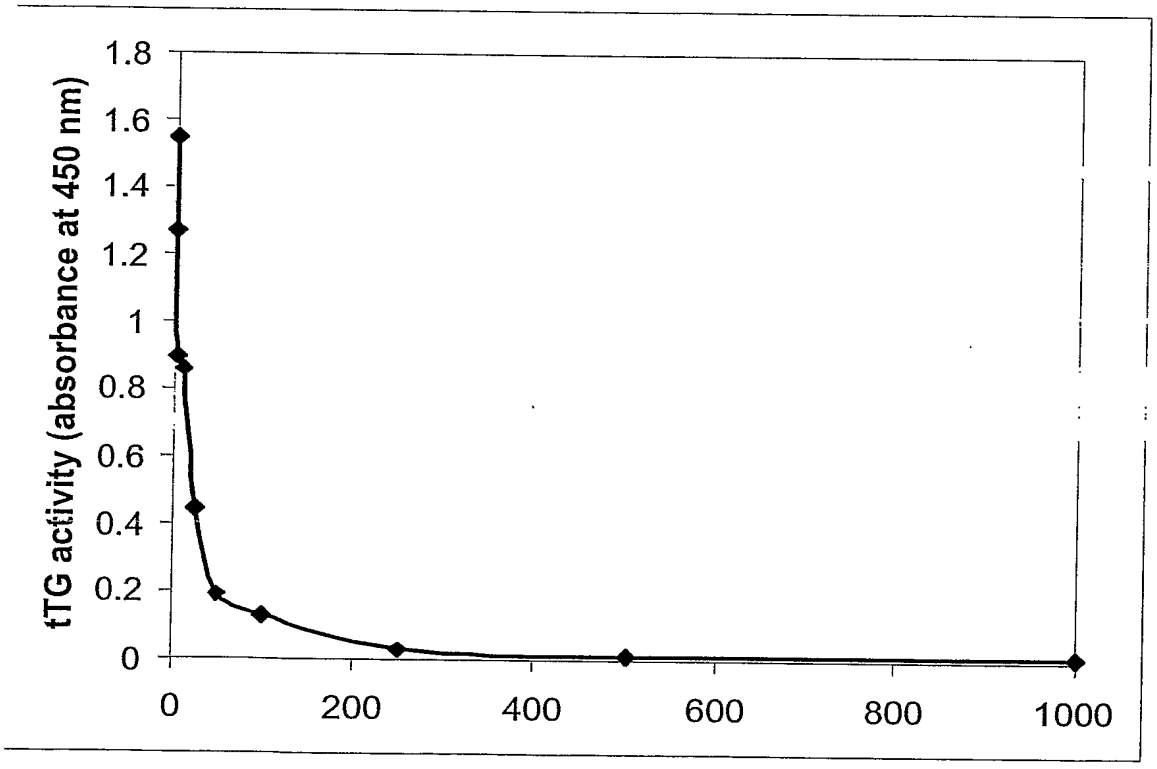

tTG activity (absorbance at 450 nm)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
200
400
600
800
1000

FIGURE 7

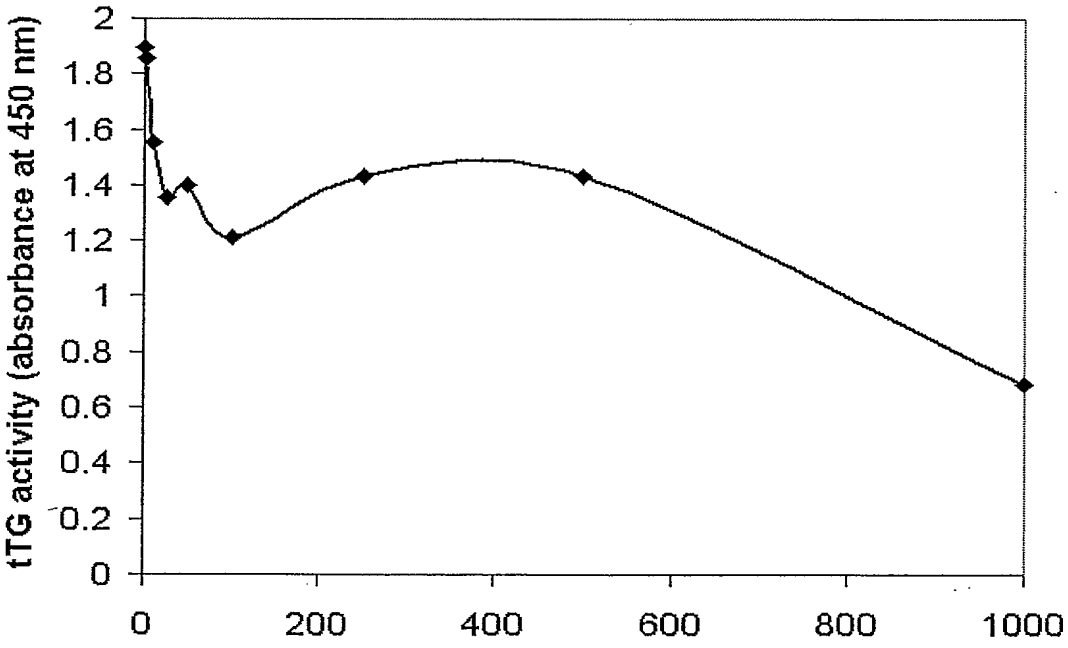

tTG activity (absorbance at 450 nm)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
200
400
600
800
1000

FIGURE 8

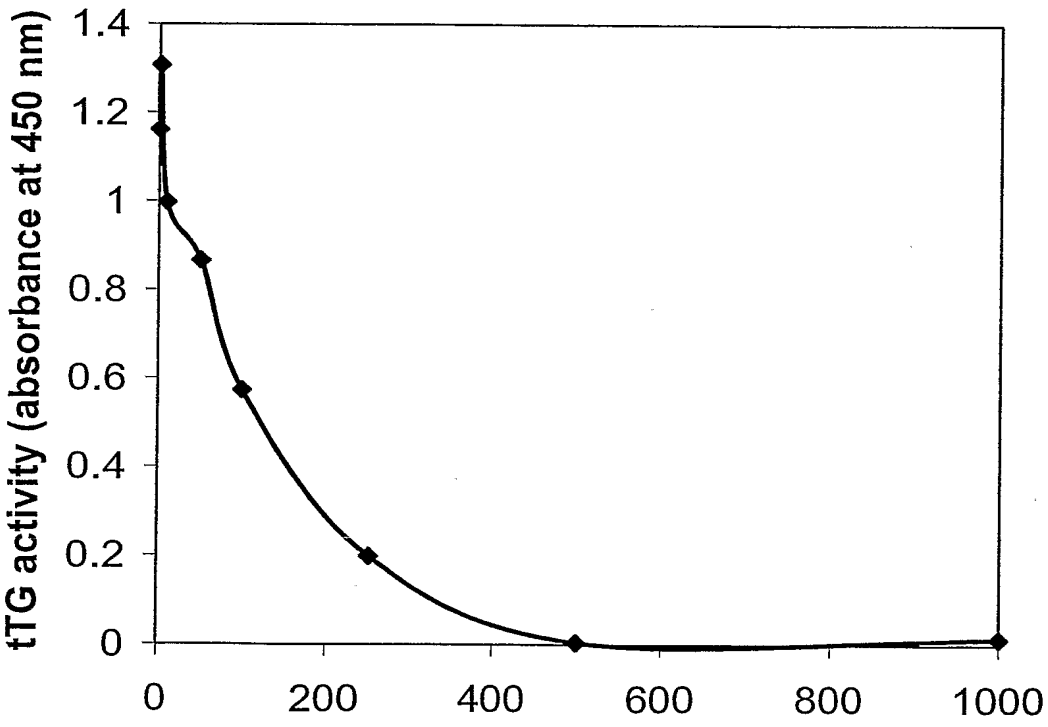

tTG activity (absorbance at 450 nm)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
200
400
600
800
1000

FIGURE 9

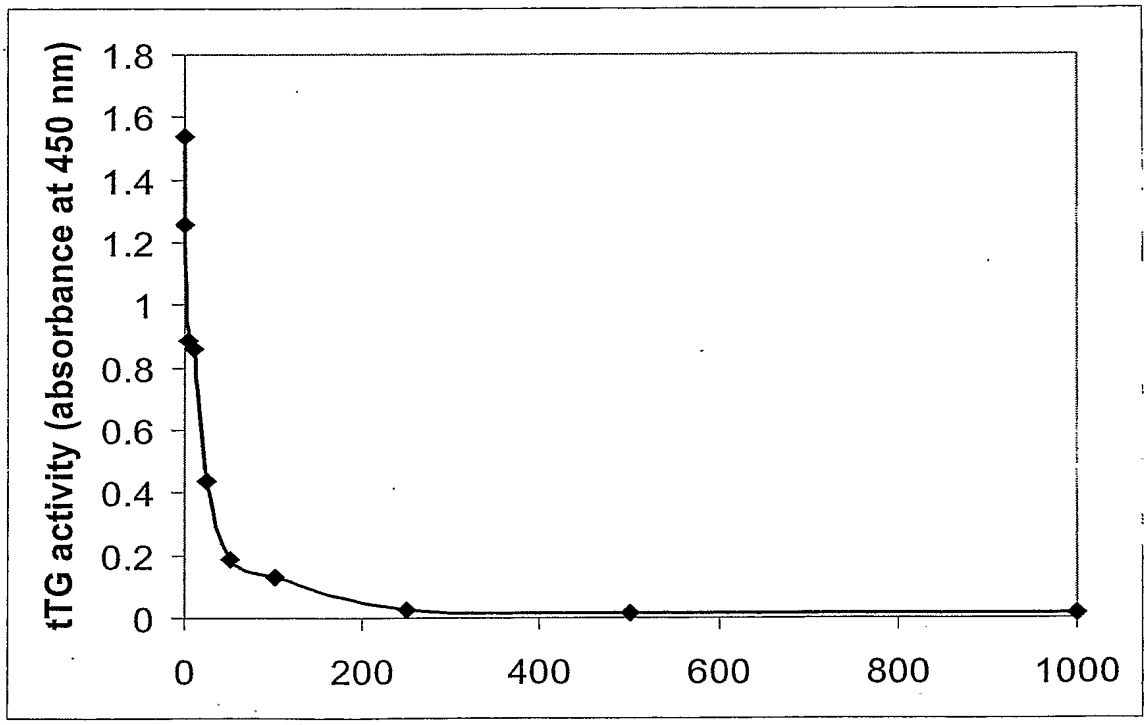

tTG activity (absorbance at 450 nm)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
200
400
600
800
1000

FIGURE 10

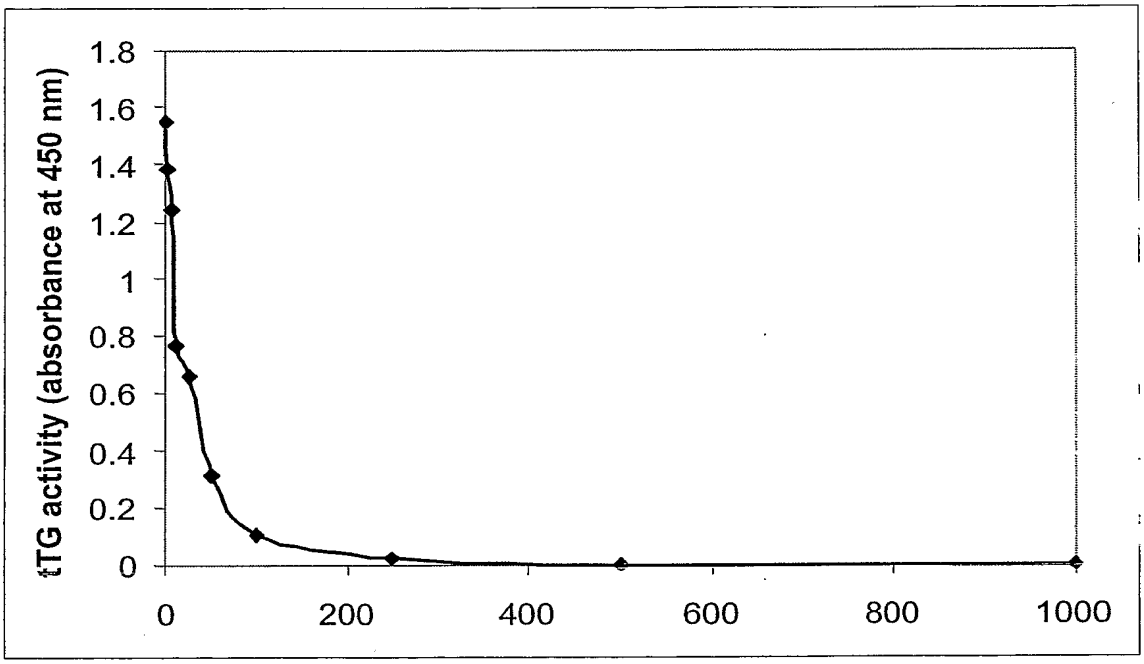
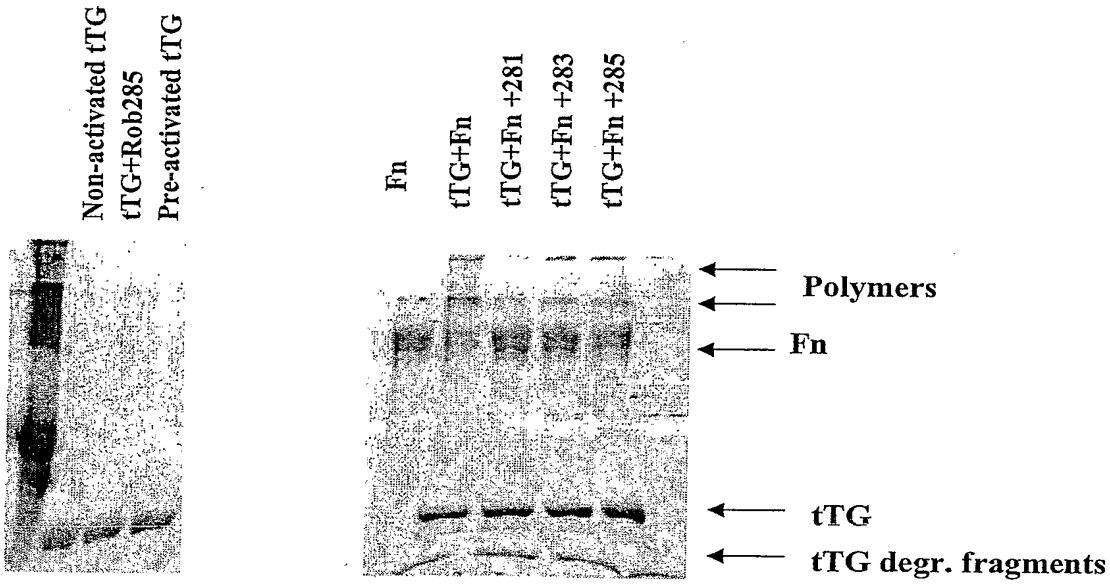

tTG activity (absorbance at 450 nm)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
200
400
600
800
1000

FIGURE 11



10ug of protein/lane

FIGURE 12(A)

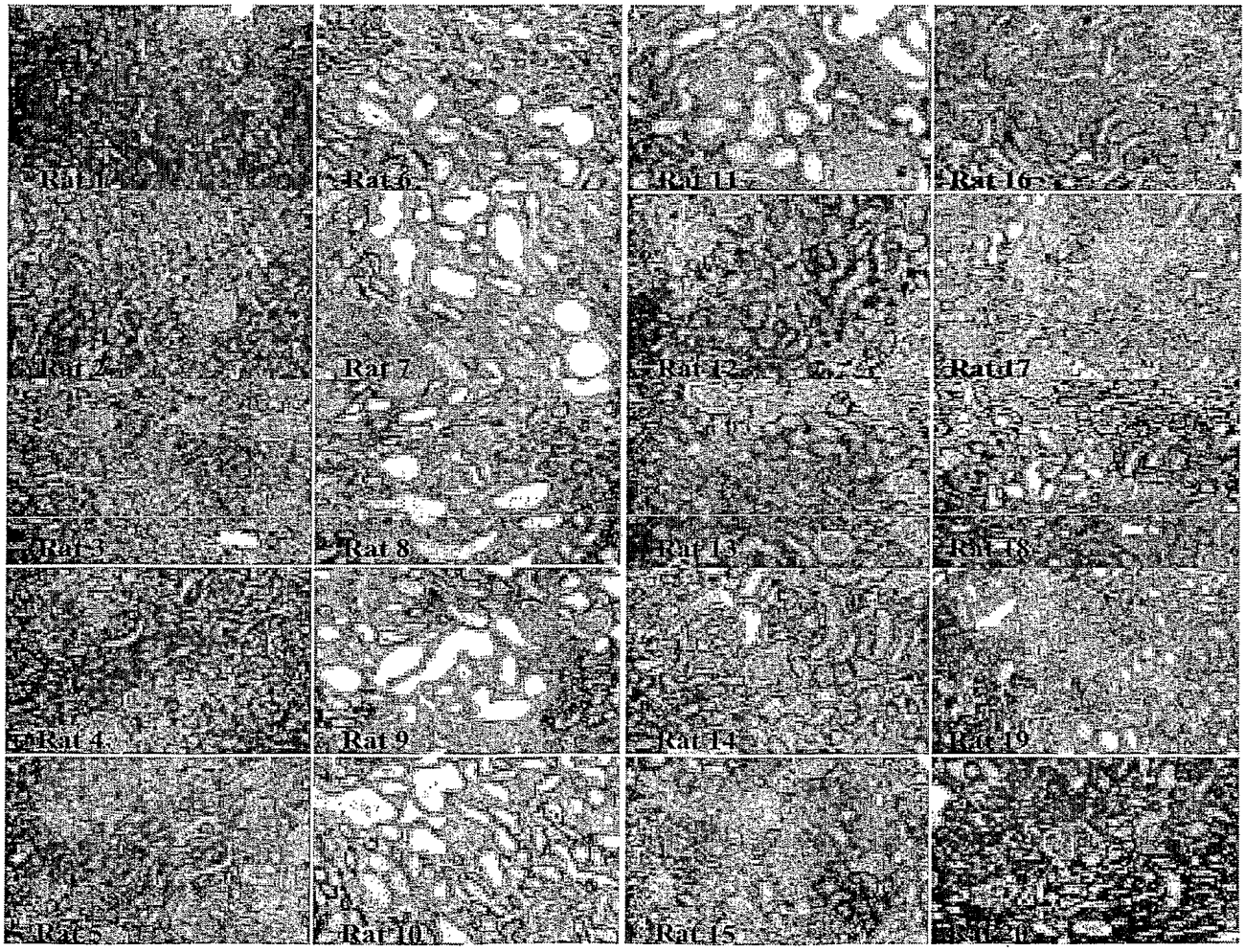

Rat 1
Rat 6
Rat 11
Rat 16
Rat 2
Rat 7
Rat 12
Rat 17
Rat 3
Rat 8
Rat 13
Rat 18
Rat 4
Rat 9
Rat 14
Rat 19
Rat 5
Rat 10
Rat 15
SNc
SNx
SNx + 281
SNx + 283

FIGURE 12 (B)

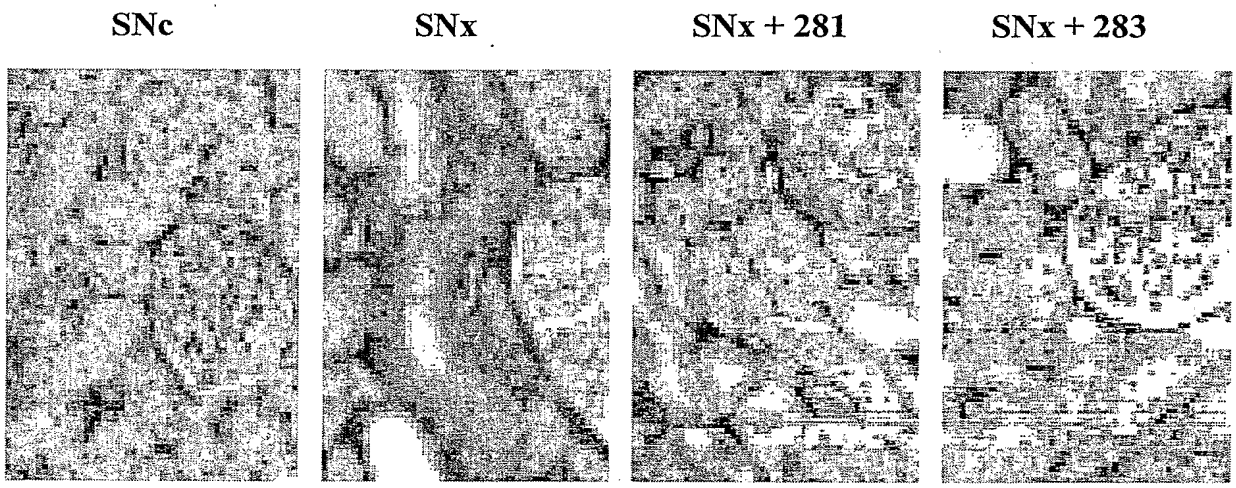


FIGURE 13(A)

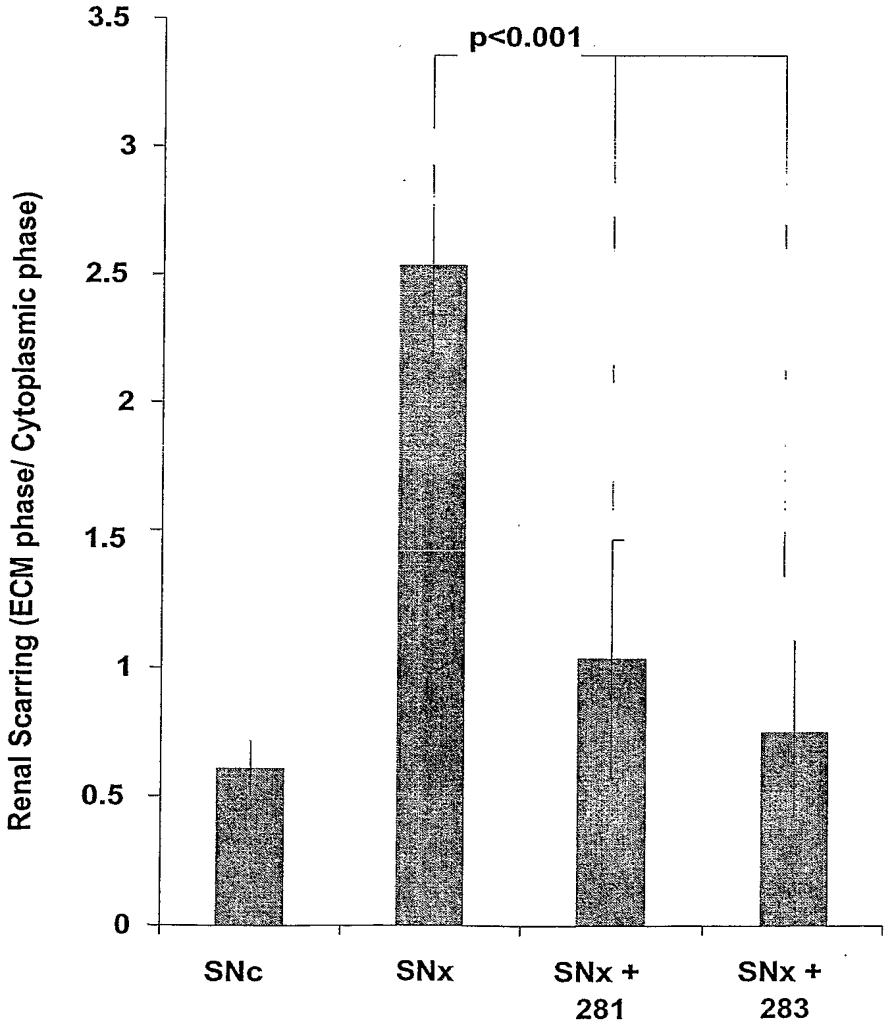

p<0.001
Renal Scarring (ECM phase/ Cytoplasmic phase)
3.5
3
2.5
2
1.5
1
0.5
0
SNc
SNx
SNx + 281
SNx + 283

FIGURE 13(B)

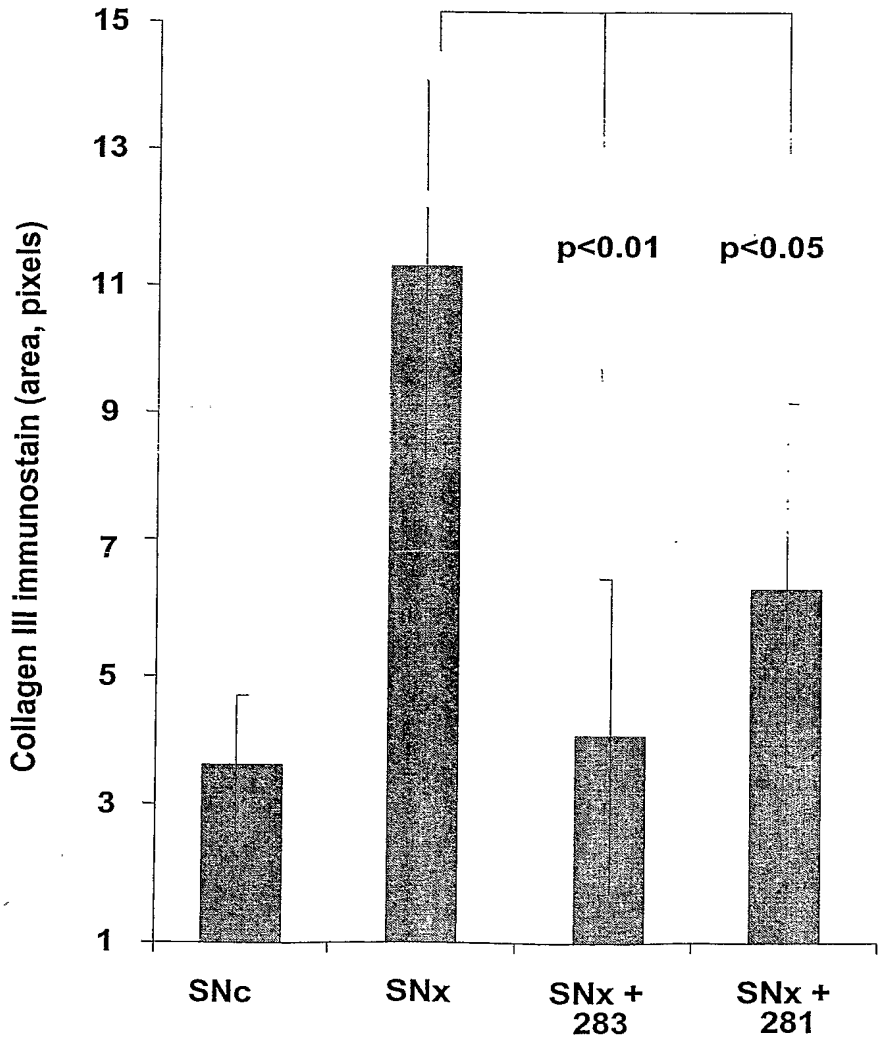

Collagen III immunostain (area, pixels)
15
13
11
9
7
5
3
1
p<0.01
p<0.05
SNc
SNx
SNx + 283
SNx + 281

FIGURE 14(A)

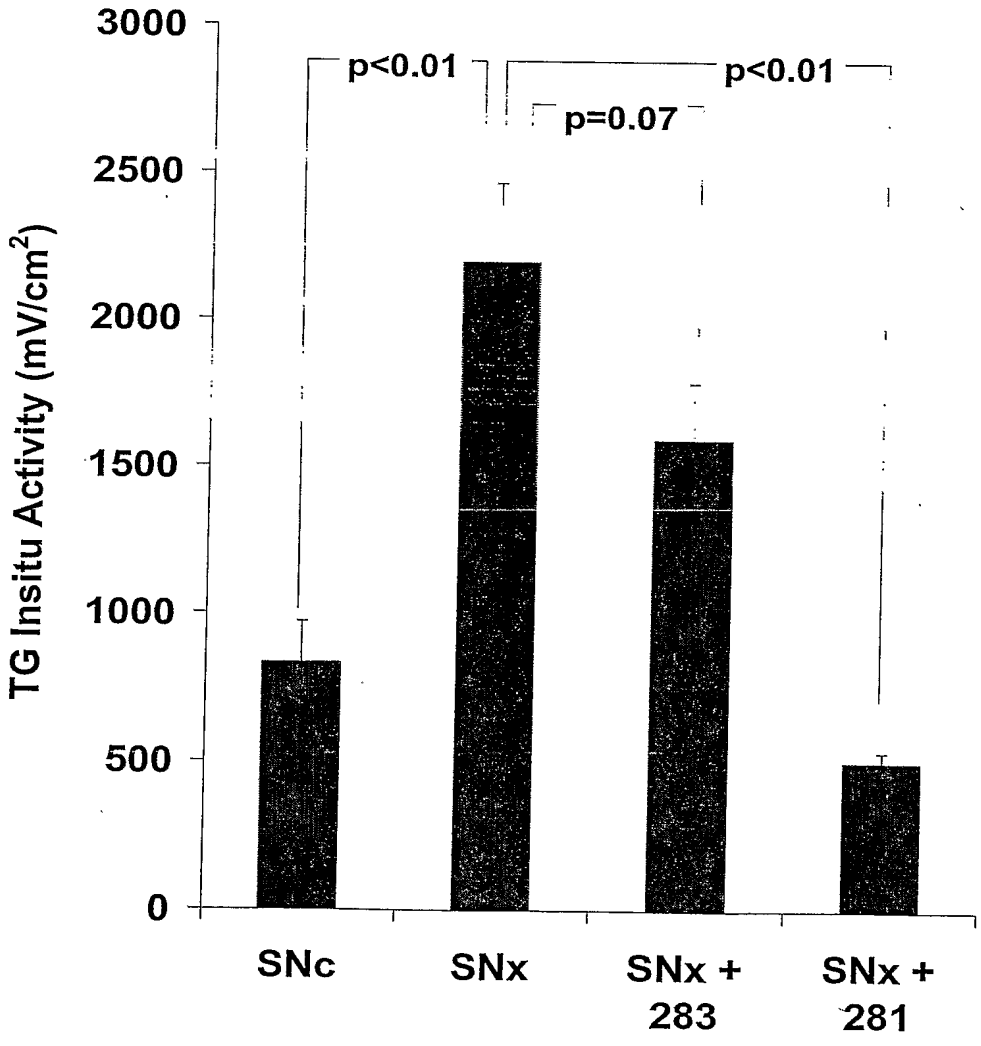

3000
2500
2000
1500
1000
500
0
TG Insitu Activity (mV/cm^2)
p<0.01
p=0.07
p<0.01
SNc
SNx
SNx + 283
SNx + 281

FIGURE 14(B)

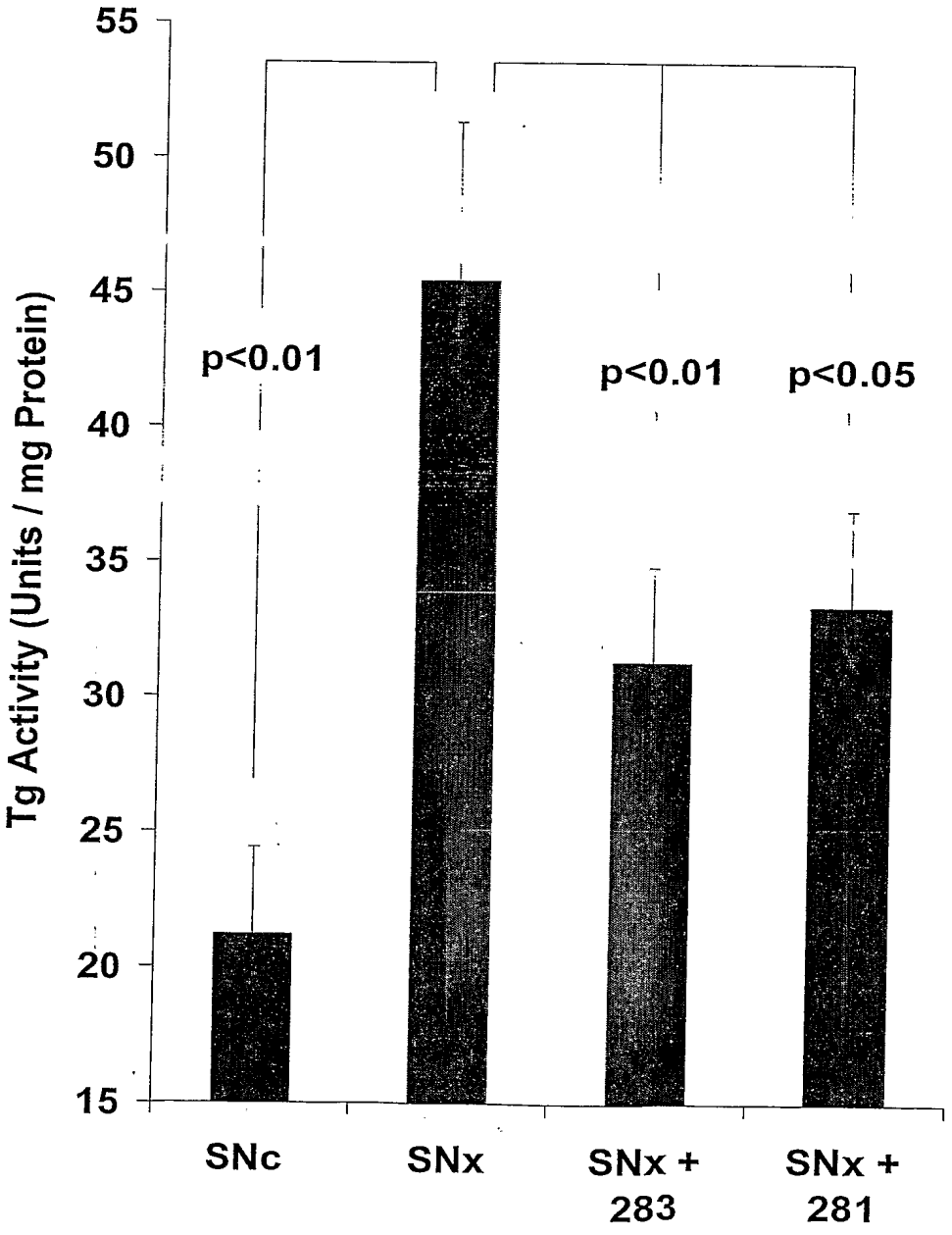

55
50
45
40
35
30
25
20
15
Tg Activity (Units / mg Protein)
p<0.01
p<0.01
p<0.05
SNc
SNx
SNx + 283
SNx + 281

FIGURE 15(A)

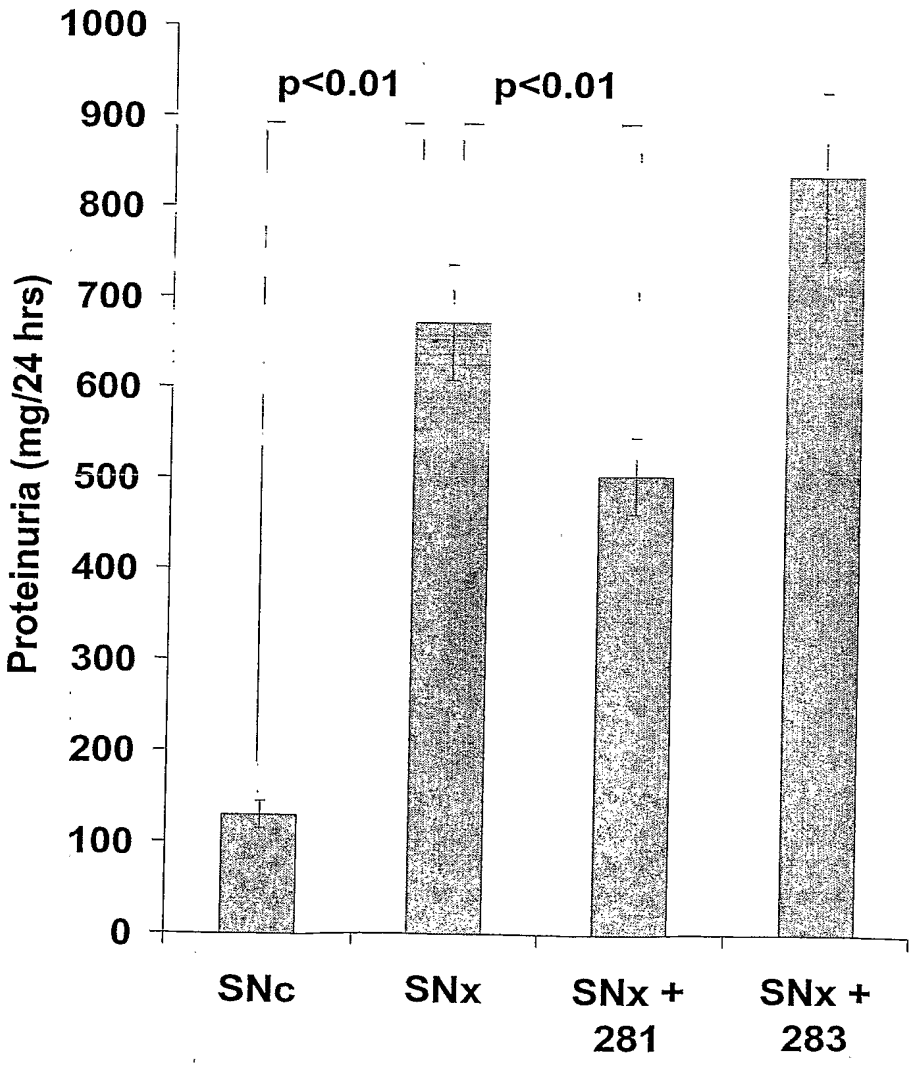
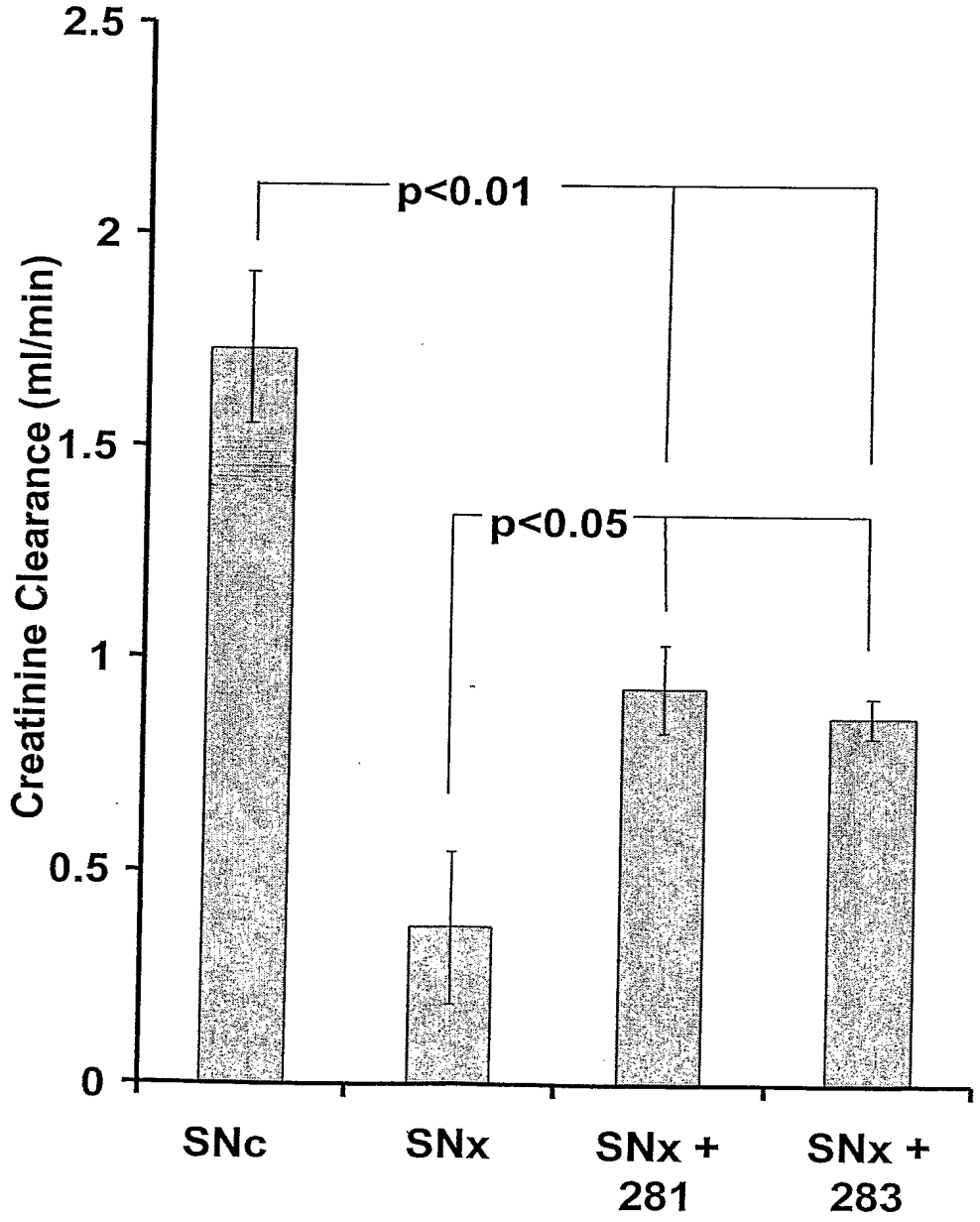

1000
900
800
700
600
500
400
300
200
100
0
Proteinuria (mg/24 hrs)
p<0.01
p<0.01
SNc
SNx
SNx + 281
SNx + 283

FIGURE 15(B)


2.5
2
1.5
1
0.5
0
Creatinine Clearance (ml/min)
p<0.01
p<0.05
SNc
SNx
SNx + 281
SNx + 283